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Review article

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Fibrosis: cross-organ biology and pathways to development of innovative drugs

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Abstract

Fibrosis is a pathophysiological mechanism involved in chronic and progressive diseases that results in excessive tissue scarring. Diseases associated with fibrosis include metabolic dysfunction-associated steatohepatitis (MASH), inflammatory bowel diseases (IBDs), chronic kidney disease (CKD), idiopathic pulmonary fibrosis (IPF) and systemic sclerosis (SSc), which are collectively responsible for substantial morbidity and mortality. Although a few drugs with direct antifibrotic activity are approved for pulmonary fibrosis and considerable progress has been made in the understanding of mechanisms of fibrosis, translation of this knowledge into effective therapies continues to be limited and challenging. With the aim of assisting developers of novel antifibrotic drugs, this Review integrates viewpoints of biologists and physician-scientists on core pathways involved in fibrosis across organs, as well as on specific characteristics and approaches to assess therapeutic interventions for fibrotic diseases of the lung, gut, kidney, skin and liver. This discussion is used as a basis to propose strategies to improve the translation of potential antifibrotic therapies.

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Introduction

Fibrosis, which is a key characteristic of a range of chronic diseases, is the consequence of a dysregulated tissue repair response following injury. Indeed, fibrosis and wound healing are orchestrated by the same cellular players[1] and share multiple mechanisms[2].

The impact of fibrosis on morbidity and mortality worldwide is substantial. However, the only approved drugs that directly target fibrosis so far are pirfenidone and nintedanib for idiopathic pulmonary fibrosis (IPF) and interstitial lung fibrosis. In addition, resmetirom, which has indirect antifibrotic activity, has recently become the first drug to be approved for metabolic dysfunction-associated steatohepatitis (MASH; previously known as non-alcoholic steatohepatitis (NASH)). On the other hand, a long list of antifibrotic compounds with a wide range of putative mechanisms have failed to show benefit in clinical trials, indicating the complex challenges faced by researchers working on the discovery and development of novel antifibrotic drugs.

With the aim of providing a guide for such researchers to address these challenges, this Review first overviews mechanisms of fibrosis, highlighting the core pathways active in fibrotic conditions across organs. We then describe specific mechanisms and characteristics of fibrotic diseases in the lung, gut, kidney, skin and liver, as well as approaches to assess fibrosis in these organs in clinical trials (Tables 1 and 2). We conclude by discussing similarities and differences in fibrotic diseases across organs (Table 3), providing proposals for strategies to improve the translation of potential antifibrotic therapies.

Fundamentals of fibrosis

Transient normal tissue repair or persistent fibrosis: what makes the difference?

Injuries initiate processes aimed at containing the damage and restoring organ function[3] (Fig. 1a). Epithelia — protective layers composed of polarized cells that are tightly coupled through intercellular junctions — are the most frequent sites of initial injury[4]. Epithelia lining different organs are typically injured in different ways as described in the organ-specific sections below, but irrespective of the cause, injured epithelial cells release fibrotic mediators, including the major profibrogenic cytokine transforming growth factor-β (TGFβ). These in turn activate local fibroblasts and macrophages, leading to the formation of fibrotic tissue characterized by excessive deposition of extracellular matrix (ECM)[5–7] (Fig. 1a).

The ECM has a key role in the mechanical stabilization of injured tissue and acts as a scaffold for the migration of reparative cells into the injury region during normal wound healing. However, in pathological fibrosis, dysregulation of ECM turnover leads to continued accumulation of ECM components such as collagen and fibronectin, disrupting tissue architecture and resulting in organ dysfunction.

Beneficial tissue repair and detrimental fibrogenesis involve essentially the same key cell players and signalling pathways, and the factors that tip the balance from normal to pathological tissue remodelling are still unclear. One difference between normal and fibrotic tissue repair is the acute versus chronic character of the injury and, thus, whether scar tissue can resolve or not. Persistent exposure to the self-generated repair environment can induce permanent epigenetic changes characteristic of fibrotic cells and/or induce programmes that render cells resistant to controlled cell death at the end of repair (reviewed elsewhere[8]). Another difference is when the severity of the damage overwhelms the capacity of the body to repair. Both chronic and severe injuries lead to an imbalance in the spatiotemporal coordination of the intercellular crosstalk that drives fibrosis[9]. Here, we focus on three key cell types for which dysregulated communication is a major factor driving acute repair into fibrosis: epithelial cells, mesenchymal cells such as fibroblasts, and immune cells, in particular, macrophages.

Epithelial cells

In the kidney, proximal tubular cells are particularly vulnerable to injury and become atrophic, with a phenotypic switch characterized by a flattened shape and expression of injury markers that include KIM1, vimentin and vascular cell adhesion molecule 1 (VCAM1), accompanied by inflammation and interstitial fibrosis[10,11]. Injured tubule epithelial cells become arrested in the G2/M cell-cycle phase and produce excessive profibrotic growth factors, leading to myofibroblast activation and fibrosis[12]. The relevance of epithelial–mesenchymal crosstalk in controlling the initiation and progression of kidney fibrosis has been shown using several techniques[13–15].

Recent seminal work has shown that injured tubular epithelial cells dedifferentiate into VCAM1$^+$ cells with a pro-inflammatory mesenchymal phenotype, which secrete cytokines and other signalling molecules that eventually cause inflammation and subsequent fibrosis[16]. This supports the theory that injured tubules are key initiators and likely crucial drivers of kidney fibrosis. An orthogonal demonstration of the key role of epithelial–mesenchymal crosstalk in driving fibrosis comes from clinical studies in which accelerated senescent tubular epithelial cells have been observed in the kidneys of patients with chronic kidney disease (CKD)[17].

Similarly, in the lung, factors that affect epithelial function result in lung fibrosis (recently reviewed elsewhere[5]). Genetic studies highlighted the variant rs35705950 of mucin 5B (*MUC5B*), expressed in distal airway epithelial cells, as being strongly associated with the risk of developing sporadic and familial IPF[18]. Genome-wide association studies (GWAS) have confirmed this finding and additionally identified several common epithelial cell structural and functional variants that increase the risk for sporadic IPF[19–22]. Selective induction of type 2 alveolar epithelial cell (AEC2, also known as AT2) apoptosis alone is sufficient to trigger the development of fibrosis[23]. Furthermore, epithelial–mesenchymal crosstalk has a crucial role in the development of lung fibrosis[24]. Selective AEC2 expression of telomere shelterin protein telomeric repeat binding factor 1 (TRF1)[25] leads to spontaneous and progressive ageing-associated lung fibrosis. Likewise, expression of a mutant form of surfactin protein C (SFTPC) in the lung epithelium drives spontaneous experimental lung fibrosis through endoplasmic reticular stress-induced apoptosis of AEC2s[26].

In the liver, epithelial (hepatocyte) injury can initiate activation of fibrotic responses in hepatic stellate cells (HSCs). For example, apoptotic bodies derived from hepatocytes activate HSCs via interactions with Toll-like receptor 9 (TLR9)[27], at least in part via inhibition of HSC chemotaxis[28]. Additional crosstalk between hepatocytes and HSCs is mediated by various damage-associated molecular patterns (DAMPs) and reactive oxygen species (ROS) generated by injured hepatocytes. Despite strong evidence for epithelial–mesenchymal crosstalk, the concept of liver epithelial–mesenchymal transition (EMT) remains controversial. Early evidence suggested that EMT of both hepatocytes and cholangiocytes contributed to liver fibrosis[29]. However, subsequent lineage tracing studies provided conflicting results[30].

Much less is known about whether the epithelium is involved in initiation and/or fibrogenesis in inflammatory bowel disease (IBD)-associated fibrosis. IL-1α (a DAMP released by multiple cell types[31])

Table 1 | Fibrosis detection methods across organs

Technology used	Comment	Use in clinical trials	Refs.
Lung			
CT	CT forms the cornerstone of diagnosis for most patients with interstitial lung disease CT is also used to assess progression of fibrosis	Central reading of CT scans to ensure enrolled subjects have the diagnosis under investigation Computer-automated image analysis of serial CT scans is increasingly being used as an end point in early-phase trials but requires validation for use in registration trials	199,209, 210,343
Histopathology	Surgical lung biopsy has a 1–2% risk of mortality. Bronchoscopic approaches are safer but are harder to interpret. For this reason, biopsy is reserved for diagnosis of individuals in whom a CT scan is truly non-diagnostic	No	209
Gut			
Endoscopy	Not able to determine fibrosis as not able to assess bowel wall transmurally	No	255
Endoscopic mucosal biopsy	Able to sample only the superficial layers of the intestine, which does not reach the submucosa	No	255
Cross-sectional imaging: MRE, CTE and IUS	Gold standard for stricture detection, but cannot accurately determine the degree of fibrosis	Yes: for morphological description of CD strictures in the terminal ileum No: for the quantification of the degree of fibrosis	255,256
Surgical histopathology	Gold standard for determination for degree of fibrosis	No: resection not feasible in all patients	255,266
Kidney			
Histopathology in kidney biopsy	Gold standard for determination of degree of fibrosis	Yes: secondary biopsies are commonly used	344
DW-MRI	The slope of change in apparent diffusion coefficient of the cortex of the kidney in DWI-MRI; no large clinical trials have been performed using this technology yet	Yes: TOP-CKD trial ongoing (NCT04258397)	279
Skin (SSc)			
Histopathological assessment of skin biopsy samples	Gold standard for research purposes rather than for routine clinical use	Skin biopsies are obtained mainly for 'omics' analyses rather than for histology	345
Ultrasound-based quantification of dermal thickness	Experimental approach that requires further validation	No	346
Clinical assessment with the modified Rodnan Skin Score	Routine clinical tool for the assessment of dermal thickness	Yes	347
Liver			
Histopathology in liver biopsy	Standardized histological scoring system; gold standard for determination of the degree of fibrosis	Yes	348
Simple fibrosis score: FIB-4, APRI	Change over time associated with advancing disease; specificity and accuracy vary in young and older populations	Likely in near future (phase I and IIa; screening for phase III trials)	326
Imaging biomarkers: MRE, VCTE	Some data show correlation with change in fibrosis, but can be confounded by the extent of necroinflammation	Likely in near future (phase I and IIa; screening for phase III trials)	326,327
Blood biomarkers: PRO-C3, ELF score, FibroTest, FibroMeter, Hepascore	Single and combination markers; varied amounts of data validating correlation with histological data	Likely in near future (phase I and IIa; screening for phase III trials)	326

CD, Crohn's disease; CKD, chronic kidney disease; CT, computed tomography; CTE, computed tomography enterography; DW-MRI, diffusion-weighted MRI; IUS, intestinal ultrasound; MRE, magnetic resonance enterography; SSc, systemic sclerosis; VCTE, vibration-controlled transient elastography.

derived from necrotic intestinal epithelial cells can activate fibroblasts and mediate intestinal inflammation[32]. IL-1α deficiency or neutralization ameliorate experimental colitis, whereas IL-1β neutralization does not[33], and IL-1α expression increases early in the course of experimental colitis, which may indicate the importance of this mechanism in fibrosis initiation[32]. Another example is epithelium-derived IL-33, which together with its receptor ST2, is increased in active IBD[34–36], and both have been shown to increase intestinal fibrosis. A single investigation suggests that mature intestinal epithelial cells transdifferentiate into mesenchymal cells by expressing typical mesenchymal cell markers as well as exhibiting mesenchymal function[37], but the degree to which this happens in patients with IBD is unclear and this finding is still to be confirmed.

Systemic sclerosis (SSc) stands out among fibrotic skin conditions, as skin fibrosis in SSc is a manifestation of a systemic autoimmune process that can also trigger fibrosis in organs such as the lungs, heart

Table 2 | Fibrosis monitoring in clinical trials: established or proposed end points

End point status	Technology/measure used	Comments	Proposed use
Lung			
Established	FVC	Provides a reproducible measure of disease severity and is the measure of choice for longitudinal assessment of disease progression	Regulator-preferred end point of choice in phase III trials
Established	Six-minute walk distance	Frequently used in trials	High variability; influenced by fitness, cardiovascular disease, pulmonary hypertension and mobility issues; often technically difficult for trial centres to perform
Proposed	CT	Image-based analysis of fibrosis progression	Multiple imaging tools are in development, but none has been adequately validated
Proposed	Blood biomarkers	Several blood-based markers that track with disease progression and that change with treatment have been identified	None has yet been fully validated
Gut			
Proposed	PRO	Recommended for use in clinical trials	Improvement in obstructive symptoms
Proposed	Stricture radiology index	Morphological evaluation of stricture	Improvement in stricture morphology
Kidney			
Established	Histopathological quantification in kidney biopsy samples	Recommended for use in clinical trials	Assessment of fibrosis progression and/or reversal
Established	eGFR slope	Recommended for use in clinical trials as fibrosis correlates well with kidney function; however, large cohorts and long follow-ups might be needed	Assessment of kidney function
Skin (SSc)			
Established	Modified Rodnan Skin Score	Frequently used in clinical trials	Assessment of dermal thickness over time and/or in response to therapy
Proposed	Ultrasound-based quantification of dermal thickness	Requires further validation	Assessment of dermal thickness over time and/or in response to therapy
Liver			
Established	Histological assessment in liver biopsy samples	Standardized histological scoring system	Staging of fibrotic injury, progression or regression of fibrosis
Proposed (limited)	Blood biomarkers; imaging biomarkers	FDA workshop in 2023 provided guidance for early-stage clinical trials and for screening for high-risk patients before liver biopsy	

CT, computed tomography; FVC, forced vital capacity; eGFR, estimated glomerular filtration rate; PRO, patient-reported outcome; SSc, systemic sclerosis.

or intestine. This systemic autoimmune response of SSc does not occur in contexts such as keloids or hypertrophic scars, in which skin fibrosis evolves as an exaggerated, localized wound healing response to injury in genetically predisposed individuals in whom it remains restricted to the skin.

In contrast to other organs discussed above, skin fibrosis in the early phases in SSc is probably not triggered by epithelial cells, but instead by injured microvascular endothelial cells[38]. Apoptosis of microvascular endothelial cells is the first detectable histopathological change in SSc[39], with insufficient clearance of these cells triggering perivascular inflammation and subsequent fibroblast activation[40]. In an attempt to maintain vascular integrity, neighbouring endothelial cells become activated and release pro-angiogenic growth factors, some of which can induce fibroblast activation and collagen deposition, for example, vascular endothelial growth factor 165 (VEGF165)[41]. Endothelial cells in fibrotic skin can undergo (partial) endothelial-to-mesenchymal transition (EndMT), with downregulation of the expression of endothelial cell markers and upregulation of fibroblast markers[42]. Microvascular injury exposes the endothelial basement membrane, which leads to platelet activation and subsequent release of profibrotic mediators from platelet granules such as platelet-derived growth factor (PDGF) or serotonin[43], which further amplify fibroblast activation and ECM deposition. Finally, microvascular damage and subsequent capillary rarefaction lead to impaired perfusion and tissue hypoxia, which stimulates ECM release from fibroblasts, in part by TGFβ-dependent mechanisms[44].

Keratinocytes, the epithelial cells of the skin, may nevertheless contribute to the progression of SSc. The pro-inflammatory and profibrotic milieu in the dermis of patients with SSc promotes an inflammatory phenotype in epidermal keratinocytes through upregulation of pro-inflammatory mediators such as IL-1α, IL-21, CCN2 and S100A9, which cross the basement membrane to amplify inflammation and to promote fibroblast activation[45–49].

Mesenchymal cells as effectors of fibrosis

One central outcome of epithelial and/or tissue injury, irrespective of the initiating cause or involved organ, is the activation of mesenchymal cells such as fibroblasts with the aim to restore organ function. A key fibroblast response in this process is the production of collagen fibre-rich ECM and its organization into mechanically resistant scar tissue to maintain the structural integrity of injured organs. However,

deposited neo-ECM can fix defects but not compensate for the loss of cells exerting organ-specific functions. For example, infectious renal glomerular fibrosis can stop the invasion of pathogens but cannot compensate for the lost nephrons. If ECM continues to accumulate after physiological repair, the ensuing fibrosis impedes normal organ function, often leading to organ failure.

Fibroblasts. Fibroblasts are connective tissue cells that encompass multiple mesenchymal cell populations that fulfil distinct homeostatic functions in healthy adult tissues, such as maintaining ECM, providing vascular support and serving as progenitor reservoirs. Fibroblastic cells include pericytes and perivascular stromal cells, mesenchymal stromal or stem cells (MSCs), fibro-adipogenic progenitors

Table 3 | Selected treatments approved or in development for fibrosis affecting various organs

Treatment type	Drug or procedure	Ref. or trial identifier
Lung		
Immunosuppression	Approved: oral mycophenolate mofetil, oral or intravenous cyclophosphamide, rituximab (CD20 inhibitor), tocilizumab (IL-6 inhibitor)	301–303,349 NCT01933334, NCT03068234, NCT03856853, NCT03221257
Antifibrotic therapy	Approved: nintedanib, pirfenidone In development: nerandomilast (PDE4B inhibitor), bexotegrast (αvβ6 and αvβ1 integrin inhibitor), admilparant (LPA1 receptor inhibitor), treprostinil (prostacyclin analogue)	139,140,142 NCT06806592, NCT04396756[136], NCT06003426, NCT04905693
Other interventions	Lung transplantation, oxygen, inhaled treprostinil (as treatment for pulmonary hypertension related to fibrotic interstitial lung disease)	205,209,350
Gut		
Immunosuppression	None	NA
Antifibrotic therapy	In development: AGMB-129 (topical ALK5 inhibitor)	NCT05843578
Other interventions	Approved: surgical resection, surgical strictureplasty, endoscopic balloon dilation	351,352
Kidney		
Immunosuppression	None	NA
Antifibrotic therapy	In development: pirfenidone, nintedanib, runcaciguat (sGC activator)	280,281
Other interventions	Approved: dapagliflozin, empagliflozin (both SGLT2 inhibitors), finerenone (mineralocorticoid receptor antagonist); multiple RAAS inhibitors (angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors) have shown positive effects on end points of kidney function but lack direct antifibrotic effects In development: zibotentan, atrasentan (both endothelin receptor antagonists), pentoxyfilline, dialysis and kidney transplantation	353–358
Skin (SSc)		
Immunosuppression	Approved: oral mycophenolate mofetil; intravenous or oral cyclophosphamide, rituximab (CD20 inhibitor); high-dose chemotherapy with haematopoietic stem cell transplantation In development: tocilizumab (IL-6 inhibitor), lenabasum (CB_2 receptor agonist), CAR-T cell therapy	301–304,359–362 151,363–368
Antifibrotic therapy	In development: fasudil, relaxin, pravastatin (all three targeting Rho–ROCK fibroblast mechanosignalling); QAX576 (IL-13 inhibitor), SAR156597 (IL-4/IL-13 inhibitor)	110 NCT00704665 NCT01268202 NCT00581997 NCT02921971
Other interventions	None	NA
Liver		
Immunosuppression	None	NA
Antifibrotic therapy	In development: simtuzumab (LOXL2 inhibitor), QAX576 (IL-4/IL-13 inhibitor)	369 NCT01266135
Other interventions[a]	Approved: resmetirom (thyroid hormone receptor β agonist) In development: GSK-233072 (ASBT inhibitor); cenicriviroc (CCR2/5 receptor antagonist); semaglutide (GLP1 receptor agonist), tirzepatide (GLP1/GIP receptor agonist); obeticholic acid (FXR agonist); lanifibranor (pan-PPAR agonist); efruxifermin (long-acting FGF21 analogue)	333,369,331,332 NCT03900429

ALK5, activin receptor-like kinase 5; ASBT, apical sodium-bile acid transporter; CAR, chimeric antigen receptor; CB_2 receptor, cannabinoid receptor type 2; CCR2; CC chemokine receptor 2; FGF21; fibroblast growth factor 21; FXR, farnesoid X receptor; GLP1, glucagon-like peptide 1; GIP, gastric inhibitory polypeptide; LPA, lysophosphatidic acid; LOXL2, lysyl oxidase-like 2; NA, not applicable; PDE4B, phosphodiesterase 4B; PPAR, peroxisome proliferator-activated receptor; RAAS, renin–angiotensin–aldosterone system; ROCK, Rho-associated protein kinase; sGC, soluble guanylate cyclase; SGLT2, sodium–glucose transporter 2; SSc, systemic sclerosis. [a]Most drugs in clinical trials for fibrotic liver diseases (in particular, metabolic dysfunction-associated steatohepatitis (MASH)) aim to reduce the metabolic and/or inflammatory impact of the disease and are not direct antifibrotics.

(FAPs) and true fibroblasts[50–52]. The distinction between these different groups is not always evident or even generally accepted[50], but is increasingly well understood with the advent of single-cell sequencing technologies (Box 1).

Fibroblast taxonomy identifies subpopulations and their molecular components that are prone to contribute to fibrosis, enabling targeting of these cells and their profibrotic functions by drug therapies. Various subpopulations of fibroblastic cells even within the same tissue are now being characterized across mouse and human organs in health and various disease states[53–60]. Fibroblast qualifiers typically used in single-cell or single-nucleus RNA sequencing (scRNAseq or snRNAseq, respectively) analysis are expression of *Col1a1* (collagen I)[61] and *Pdgfra*, which encodes the PDGF receptor PDGFRα[62], *Dpt* (encodes dermatopontin)[63] and *Tcf21* (ref. 64), in addition to more generic mesenchymal markers such as vimentin[65], *Gli1* (ref. 66), hypermethylated in cancer 1 (*Hic1*)[67], CD90 (*Thy1*)[68] and fibroblast-'specific' protein 1 (*Fsp1*, also known as *S100a4*)[69].

Although the power of scRNAseq and snRNAseq is undeniable, care must be taken to not overinterpret their findings. First, fibroblastic cells, in particular, in fibrosis, are embedded in dense connective

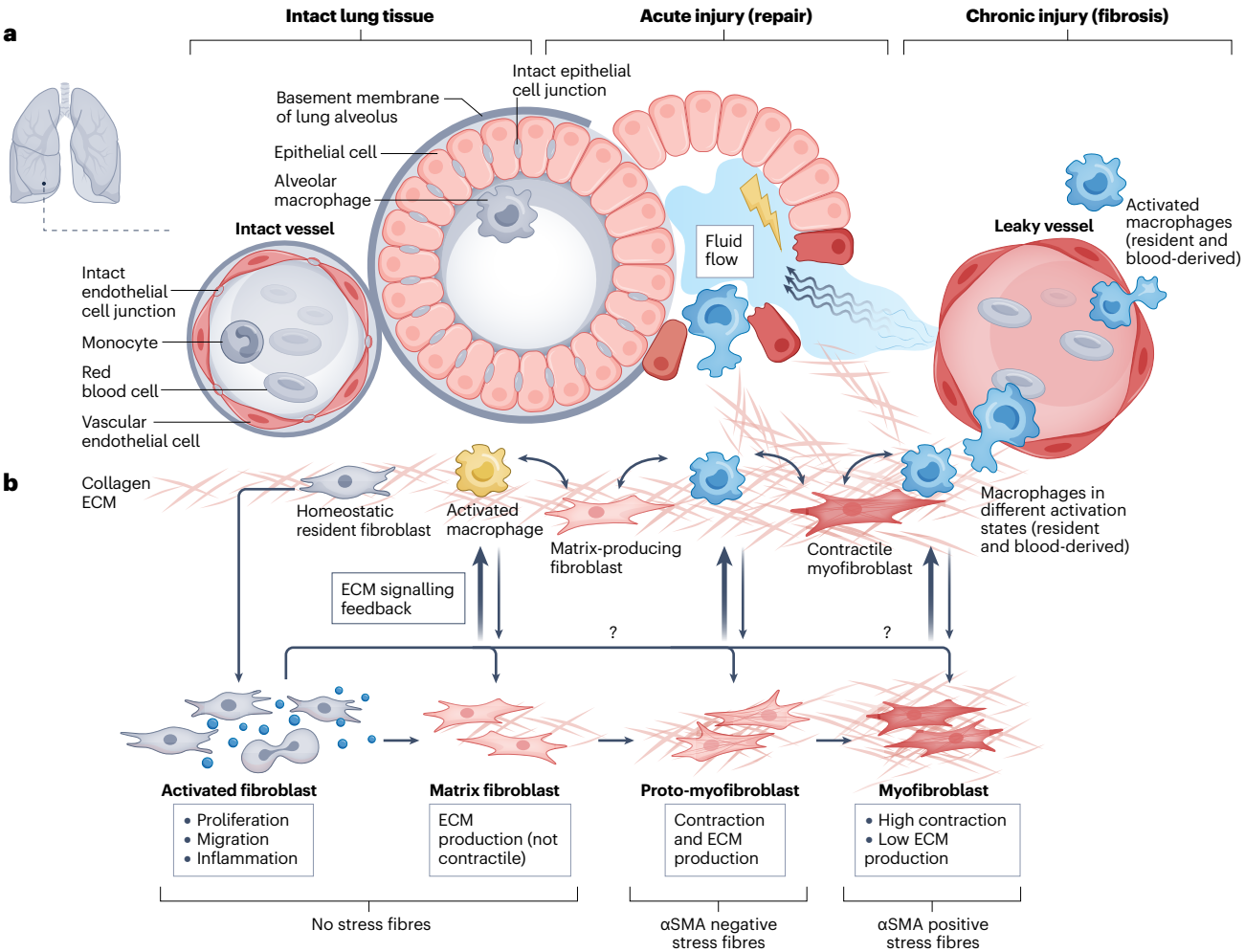

Fig. 1 | Initiation of fibrosis. a, Onset of fibrosis upon epithelial injury. Both normal and fibrotic tissue repair typically start with an injury to the epithelial lining of organs, here exemplified by lung alveolar epithelium. Injury to epithelial cells (indicated by the lightning symbol) — for example, owing to genetic polymorphisms, autoimmune disease or exposure to environmental toxins — compromises tissue architecture and barrier function, which results in local mechanical stresses that arise from enhanced fluid flow and cell strain. Epithelial cell-derived injury signals activate local and circulating monocytes and/or macrophages (centre of the figure) and fibroblasts (left) into various repair phenotypes. Persistent crosstalk between such activated cells (curved arrows) can result in the formation of profibrotic niches that drive the fibrotic process. **b**, Fibroblastic cell activation states and functions. Mesenchymal cells with various homeostatic functions and locations in normal tissue are activated by tissue injury to contribute to acute repair by proliferating, migrating into the injury site and performing basic inflammatory functions. Either directly or by passing over these initial activation states (it is not currently clear which), activated matrix fibroblasts produce collagen extracellular matrix (ECM) to restore lost tissue architecture. The low traction forces of migrating fibroblasts lead to collagen fibre alignment and enhanced mechanical resistance of the scar ECM, resulting in the formation of actomyosin bundles. Initially, these so-called stress fibres are composed of cytoplasmic β- and γ-actins in contractile proto-myofibroblasts that further contribute to ECM remodelling and stiffening. With increasing ECM stiffness, proto-myofibroblast stress fibres progressively incorporate neoexpressed α-smooth muscle actin (αSMA), which confers even higher contractile activity to overcome higher ECM stiffness.

tissues and may be under-represented in conventional cell-isolation protocols[58]. Second, studies often enrich fibroblastic cells by flow sorting based on one of the above-noted qualifiers, such as *Pdgfra*[63] or *Col1a1* (ref. 58), which can potentially bias the results; and third, fibroblasts are highly plastic cells. Despite having distinct transcriptional profiles and specialized functions in normal tissues, all fibroblastic cells appear to be activated by injury signals towards common phenotypes, most notably inflammatory or scar-making properties[70] (Fig. 1a). It is still a matter of debate whether these functions are performed consecutively by a particular cell type or whether different fibroblast subpopulations split these two tasks over the course of healing[63,71,72]. Consequently, instead of representing different fibroblast types, distinct RNA profiles may instead signify fibroblast functional states, such as proliferation, immune functions, migration, ECM production and contraction. Because all these functions cannot be performed simultaneously for sheer energetic reasons, fibroblast functional specialization will necessarily reduce versatility. For instance, expression of collagen triple helix repeat-containing 1 (CTHRC1) emerges as a marker for ECM-producing fibroblasts in different fibroblastic conditions; CTHRC1-positive fibroblasts are characterized by enhanced ECM production but seem to lack contractile cell markers such as α-smooth muscle actin (αSMA)[58,73].

Myofibroblasts. Two repair functions that are traditionally considered to coexist in activated fibroblastic cells are ECM production ('fibro') and contraction ('myo') of the neo-ECM into a stiff resistant scar. Consequently, these cells have been named myofibroblasts, and the process of becoming a myofibroblast is termed fibroblast-to-myofibroblast activation, or in brief, myofibroblast activation[74].

'Myofibroblast' is a functional definition and there are few if any unique molecular markers that allow discrimination of myofibroblasts from other fibroblastic cells. To add the 'myo' prefix to an ECM-associated fibroblastic cell, the presence of actin–myosin bundles resulting in the ability to exert contractile force is required[75,76] (Fig. 1a). Notably, all fibroblastic cells reorganize actin into contractile bundles within minutes of contact with stiff surfaces, making this a challenging in vitro fibroblast activation readout. Stress fibres and contraction are also difficult to assess with routine diagnostics, and molecular markers need to be used in immunohistochemistry and/or RNA sequencing approaches[65]. The most reliable and widely used myofibroblast marker is neo-expression of αSMA, and its incorporation into stress fibres augments fibroblast contractile activity. Other myofibroblast activation markers are the ECM proteins extradomain-A fibronectin, periostin, tenascin C, latent TGFβ binding protein 1 (LTBP1), CTHRC1 and connective tissue growth factor (CTGF, which is not a growth factor and was renamed CCN2; reviewed in ref. 77). Surface markers for myofibroblasts potentially serving as homing receptors for drugs or to identify myofibroblasts in flow cytometry are scarce, and α11β1 integrin, cadherin 11 and THY1 have been suggested for these purposes[77]. However, none of the above listed markers is unique to myofibroblasts, and marker combinations are required to conclusively discriminate from other cells.

It largely remains a mystery why myofibroblasts are cleared at the end of physiological healing but persist in fibrosis and therefore create the debilitating tissue contractures characteristic of fibrotic disease in all organs[55,78–84]. Current concepts explain myofibroblast persistence in fibrosis by the chronic presence of injury signals, longevity of myofibroblasts by becoming either senescent or resistant to apoptosis[8], epigenetic manifestation of myofibroblast traits (reviewed elsewhere[77,85]) or autocrine activation through a self-generated myofibroblast environment[86]. One central element driving this positive feedback loop of myofibroblast activation is the mechanical state of the ECM.

Myofibroblast ECM production and contraction cause tissue stiffening, which in turn promotes accumulation of myofibroblasts from their various precursors and then maintains their activity. Consequently, therapeutic strategies to interfere with fibroblast mechanosensing and mechanotransduction mechanisms are a reasonable proposition[87]. Physical organ injury, such as skin trauma or external ventilation-induced lung damage, destroys the fibrous ECM architecture that normally protects fibroblastic cells from strain in these mechanically challenged organs[88–90]. Once this buffer function of the ECM is lost, tissue-resident cells are directly exposed to the stresses, for instance, during expansion of inhaling lungs or the cardiac diastole. Formation of stress fibres and activation of ECM secretion programmes in this instance are a rapid response to prevent further injury[91] (Fig. 1b). Although we focus here on the effect of fibroblast activities on ECM mechanical properties and vice versa, the same fundamental mechanosensing and mechanotransduction mechanisms apply to other cells in the fibrotic environments, such as epithelial cells and immune cells. Recent reviews provide more in-depth discussions on the specific receptors and cytoskeletal elements used by these cells[92–94].

The main cell surface receptors to sense altered ECM stress are transmembrane integrins[91]. The same integrins also transmit stress fibre-generated contractile forces to the ECM at sites of so-called focal adhesions. Hence, they are ideally positioned to adapt fibroblast activities to the progress of ECM remodelling during repair. Consequently, integrins enriched in activated myofibroblasts such as α11β1 integrin and possibly α1β8 integrin are being tested as antifibrotic drug targets in various experimental models[95,96]. Other potential drug targets are structural and signalling proteins that link to integrins and stress fibre actin in ECM adhesion complexes, such as focal adhesion

Box 1 | Single-cell studies of fibrosis

Single-cell RNA sequencing (scRNAseq) and single-nucleus RNA sequencing (snRNAseq) approaches with combined protein and DNA accessibility measurement, and more recently spatial transcriptomics, have enabled discoveries about the pathogenesis of fibrosis in various organs[54,249,370–372]. These technologies have provided new insights into fibroblast, myofibroblast and macrophage heterogeneity, functional states and differentiation trajectories upon injury[63,271,373], allowed the resolution of cell–cell interactions and enabled activated pathways to be mapped spatially in situ on a single-cell level and for gene-regulatory networks[374–376]. For instance, IL-36γ-producing macrophages have been reported to drive IL-17-mediated fibrosis[377] and resident BHLHE41^{+} macrophages have been demonstrated to transiently suppress myofibroblast activation after myocardial infarction[378].

Although snRNAseq has a higher RNA amplification bias owing to lower levels of RNA around and within the nucleus, the major advantage is that it can be performed on frozen tissue and the cell-type isolation bias is probably reduced compared with enzymatic dissociation[379]. This specific feature of snRNAseq makes it currently the gold standard to analyse large tissue repositories. These technologies promise to fuel drug discovery and precision medicine in fibrotic diseases across organs[380].

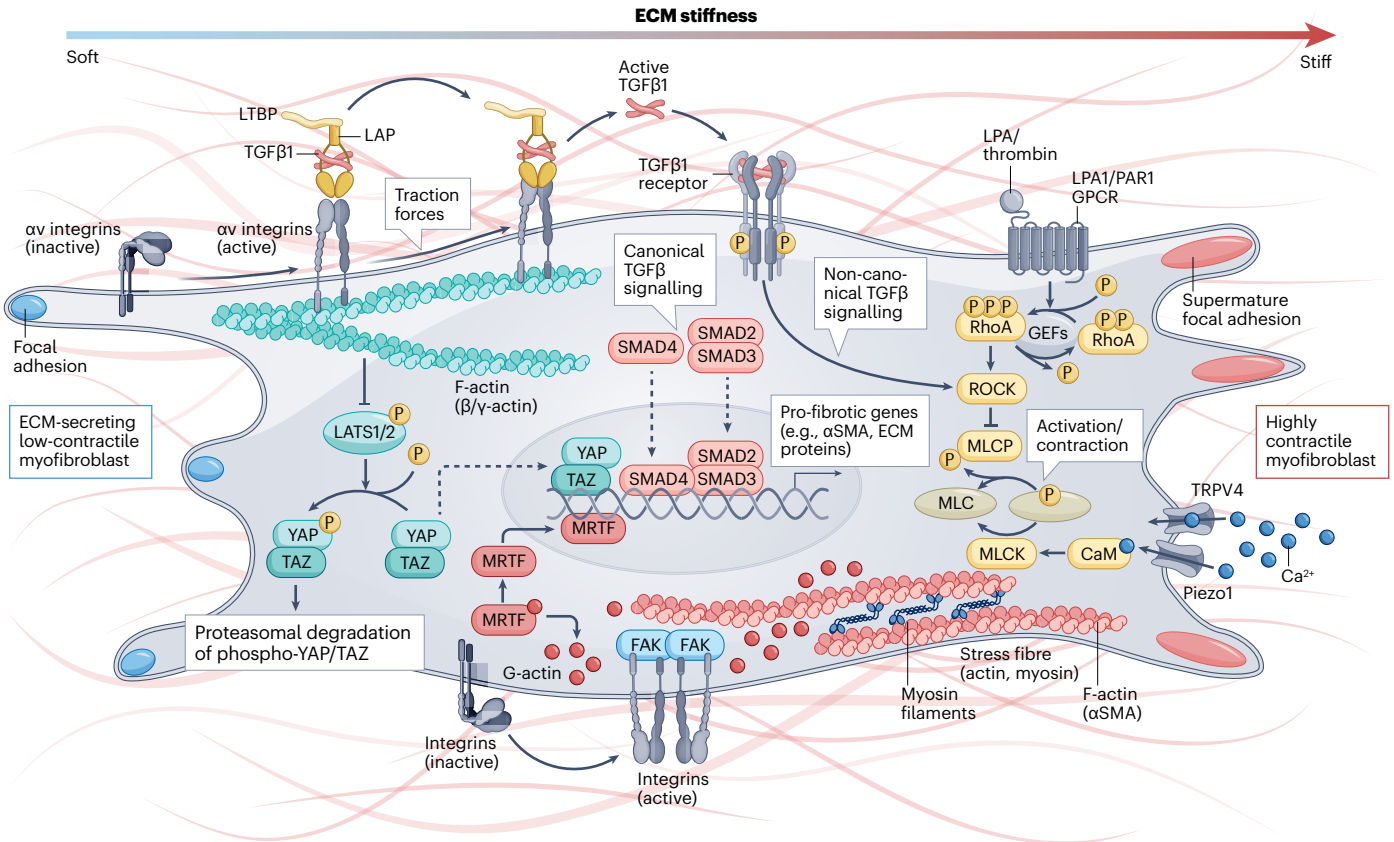

Fig. 2 | Myofibroblast activation states and pathways. Key pathways that regulate fibroblast-to-myofibroblast activation, described from left to right of the figure. Fibroblastic cells secrete transforming growth factor-β1 (TGFβ1) in complex with its latency-associated pro-peptide (LAP), which can bind to latent TGFβ1 binding proteins (LTBPs) in the extracellular matrix (ECM). Stiff fibrotic ECM resists cell forces transmitted to LAP via αv integrins that mechanically liberate active TGFβ1. TGFβ1 binding to its receptor triggers canonical SMAD signalling, driving the transcription of profibrotic genes. Another TGFβ1 signalling pathway is activation of the Rho–Rho-associated protein kinase (ROCK), which is also mediated through G protein-coupled receptors (GPCRs) upon binding of lysophosphatidic acid (LPA) and/or thrombin. GPCR activity produces active (GTP-bound) RhoA by regulating guanidine nucleotide exchange factors (GEFs). Active RhoA–ROCK drive fibroblast contraction by inhibiting myosin light chain (MLC) phosphatase (MLCP) and activating myosin light chain kinase (MLCK). MLCK is also activated by cytosolic calcium elevations; for instance, following stretch-induced opening of Piezo1 or transient receptor potential vanilloid 4 (TRPV4) ion channels. Additionally, activation of integrin signalling — for example, through focal adhesion kinase (FAK) — results in the polymerization of globular into filamentous (F-) actin. High levels of F-actin and active RhoA inhibit the Hippo signalling components large tumour suppressor kinases LATS1 and LATS2, ultimately resulting in dephosphorylation and nuclear translocation of Yes-associated protein 1 (YAP) and PDZ-binding motif (TAZ) co-transcription factors, whereby they drive the transcription of profibrotic genes; phosphorylated YAP and TAZ undergo proteasomal degradation in the cytosol. Furthermore, actin polymerization releases myocardin-related transcription factor A (MRTFA) from bound G-actin to translocate to the nucleus and drive profibrotic gene products. Although SMADs, YAP/TAZ and MRTFA all regulate the transcription of profibrotic genes, they occupy different promoter regions and engage different co-transcription factors. αSMA, α-smooth muscle actin; P, phosphate.

kinase (FAK), talin 1, kindlin 2 and integrin-linked kinase (ILK, which — ironically — does not link to integrins and has no kinase activity)[90,97–100]. However, these components are common to all adhesive cells, and specific targeting of myofibroblasts is a challenge.

Mechanosensors other than integrins include the hyaluronic acid receptor CD44 (ref. 101), the fibrillar collagen-binding discoidin domain receptor DDR1 (refs. 102,103) and the stretch-activated membrane channels transient receptor potential vanilloid 4 (TRPV4)[104,105] and Piezo1 (refs. 106,107). Function blockade of TRPV4 using the inhibitor GSK2798745 was effective in reducing mechanical myofibroblast activation and bleomycin-induced mouse lung fibrosis[108], but data on Piezo1 modulation are not available. Common downstream signalling events of TRPV4 and Piezo1 stretch channels increase cytosolic Ca^{2+} and Rho–Rho-associated protein kinase (ROCK) signalling, both resulting in enhanced actin–myosin activity[87,91] (Fig. 2). These pathways are central in regulation of physiological contraction of a multitude of other cells, such as cardiac, skeletal and smooth muscle cells, and so their value as specific anti-myofibroblast targets is debatable[109]. Nevertheless, the ROCK inhibitors fasudil and relaxin were shown to reduce organ fibrosis and wound contraction[110] (Table 3). Myofibroblast-specific targeting of Rho–ROCK signalling is possible at the level of upstream G protein-coupled receptors (GPCRs) and their ligands, such as the lysophosphatidic acid (LPA) receptor[111,112], the dopamine D_1 receptor[113] and the oestrogen GPCR[114].

Multiple myofibroblast downstream mechanosignalling events converge with other profibrotic pathways at the level of myocardin-related transcription factor A (MRTFA; also known as MKL1), transcriptional coactivator with PDZ-binding motif (TAZ) and Yes-associated protein 1 (YAP)[115] (Fig. 2). For all three, YAP, TAZ and MRTFA, high mechanical cell stress promotes their translocation to the nucleus as co-transcription factors that drive expression of fibrosis-related genes, including the regulation of each other[116–118]. When cytosolic non-polymerized G-actin levels drop upon actin polymerization into stress fibres, previously actin-bound MRTFA is free to travel into the nucleus, where it enhances gene transcription (Fig. 2). Systemic delivery of MRTF signalling inhibitors, such as CCG-203971, CCG-100602 and CCG-222740, alleviates development of murine fibrosis of the pancreas, gut and eye[119–121].

Cytosolic dephosphorylation by the binding factor large tumour suppressor kinase 1 (LATS1) and/or LATS2 allows YAP/TAZ nuclear translocation to enhance gene transcription[117]. Although YAP and TAZ are often used synonymously and share common functions, they are also distinctly regulated for expression, nuclear import and nuclear retention[122,123]. Inhibition of YAP and TAZ using verteporfin reduced accumulation of myofibroblasts and scar tissue in animal models of organ fibrosis[124,125]. Several statins recently emerged from high-throughput screens with small-molecule libraries as possible regulators of YAP signalling and drugs that target the mevalonate pathway in human lung fibroblasts[126]. In addition, inhibitors of GPCRs upstream of YAP signalling have been shown to be effective in treating experimental lung fibrosis[113].

The mechanical state of the ECM is tightly linked to the availability of TGFβ1. The TGFβ1 pro-peptide, called latency-associated pro-peptide (LAP), remains non-covalently bound to TGFβ1 after intracellular cleavage and secretion[127,128]. Covalent attachment of latent TGFβ1 to a larger complex in the ECM provides mechanical resistance to fibroblastic force transmission through a specific set of αv integrins that bind to an Arg-Gly-Asp (RGD) binding site in LAP[129–132]. Forced unfolding of the latent complex liberates active TGFβ1 in a process that is exquisitely dependent on the remodelling state (that is, mechanical resistance) of the ECM[129,133] (Fig. 2).

Although global inhibition of active TGFβ1 failed in clinical trials owing to severe side effects, such as exacerbated inflammation and epithelial growth, specific inhibition of TGFβ1-activating integrins is a promising strategy to target the profibrotic actions of TGFβ1 (ref. 134). Specific antibodies and small-molecule inhibitors directed against the TGFβ1-activating fibroblast integrins, most notably αvβ1 integrin, effectively reduced fibrosis in several organs in animal models[135], and antifibrotic effects have been reported for bexotegrast (also known as PLN-74809), an oral small-molecule inhibitor of the αvβ6 and αvβ1 integrins, in a phase IIa trial for IPF[136] (Table 3). However, the sponsor has very recently discontinued a phase IIb trial (NCT06097260) of bexotegrast in IPF, following a prespecified data review and recommendation by the trial's independent data safety monitoring board (see the press release in Related links). A phase IIb study (NCT01371305; see ClinicalTrials.gov) of a monoclonal antibody known as BG00011 (formerly STX-100) directed against the epithelium-specific TGFβ1-activating integrin αvβ6 was also terminated early owing to safety concerns. A phase II trial of abituzumab (a monoclonal antibody directed against αv integrin that has shown promising safety and tolerability profiles in clinical trials for cancer[137,138]) involving patients with SSc-associated interstitial lung disease (SSc-ILD) (NCT02745145) was also terminated prematurely, in this case owing to difficulties in recruiting eligible patients.

Only two drugs, nintedanib and pirfenidone, are currently approved to treat pulmonary fibrosis and lung fibrosis in SSc[139]. The biological mode of action of these two drugs is not known other than that they are rather broad inhibitors of various kinases downstream of profibrotic signalling pathways[140–142]. Cytokines and receptors best studied for their profibrotic effects on fibroblasts include IL-4, IL-6, IL-11, IL-13, angiotensin II, endothelin 1, PDGF, LPA and TLR4 (refs. 83,143–146).

The inverse approach to blockade of profibrotic cytokine signalling is treatment with cytokine ligands or synthetic agonists of receptors that mediate antifibrotic signalling. For instance, therapies based on the antifibrotic fibroblast growth factor 2 (FGF2) are envisioned to reduce fibrosis, as supported by mouse and culture models of pulmonary and dermal fibrosis[147,148]. More recently, the cannabinoid receptors CB_1 receptor and CB_2 receptor were found to be highly upregulated in skin fibroblasts of patients with SSc[149]. Consistent with the known anti-inflammatory actions of cannabinoids, treating cultured SSc fibroblasts with the CB_1 receptor agonist WIN55,212-2 was shown to reduce ECM production, myofibroblast activation and TGFβ expression[150]. Treatment with the CB_2 receptor agonist lenabasum was shown to improve diffuse cutaneous SSc in phase II trials, and reduced pro-inflammatory and profibrotic gene expression in skin biopsy samples[151] (Table 3).

Of the cytokines considered profibrotic with respect to their myofibroblast-promoting actions, IL-11, a member of the IL-6 family, is the most recent addition. Inhibition of IL-11 protects against heart, lung and kidney fibrosis in animal models[152–154] and this intervention is currently being investigated in phase I trials. The type 2 cytokines IL-4 and IL-13 are best known for their induction of TGFβ production and other profibrotic actions of macrophages[155,156], but IL-13 has also been suggested to directly stimulate fibroblastic cells to produce collagen[157]. Neutralizing antibodies and inhibitors of IL-13 and IL-4 receptors reduced fibrosis in experimental pancreatitis and MASLD, and in a clinical trial for the treatment of pulmonary fibrosis, SSc and keloids[158,159] (Table 3). Alternatively, instead of inhibiting single growth factors that promote fibroblast activation, one can also envision inhibiting the formation of the cells that make these factors — macrophages[113].

Macrophages: where the cytokines come from

The control of fibroblast activation is not only dependent on the mechanical conditions provided by the ECM, but is also intricately linked to their inflammatory environment. Of the various immune cells that contribute to normal and pathological organ repair, we focus on macrophages and how their crosstalk with fibroblastic cells drives myofibroblast activation[70,160,161]. Macrophage recruitment and activation from bone marrow-derived circulating monocytes or tissue-resident macrophages is the normal inflammatory response to organ injury[162]. The conventional view on how macrophages act on fibroblast activation is that it is by paracrine signalling through cytokines. For instance, macrophage-derived FGFs, PDGFs, VEGFs, IL-6, IL-13, TGFα and TGFβ1 all contribute to normal healing[163–167]. Like fibroblasts, macrophages become a problem and drive fibrosis when they persist past normal repair, continue to be drawn into the fibrotic tissue in secondary waves or become unduly activated (also known as polarization)[163,168–174].

Single-cell genomics studies have provided insights into immune cell heterogeneity, their specific profiles during tissue fibrosis and the connections of inflammation and fibrosis. Recent data suggest an important role of monocyte-derived macrophages in fibrosis across major organs. These macrophages have been termed scar-associated macrophages or $SPP1^+$ macrophages. $SPP1^+$ macrophages are more

abundant in fibrotic versus non-fibrotic regions in IPF[175], as well as in hepatic fibrosis[176,177]. Machine learning analyses of spatial transcriptomics data in human myocardial infarction identified that the presence of these macrophages always predicted the presence of myofibroblasts and vice versa[54]. This spatial dependency suggests crosstalk of the two cell types and probably a mechanism whereby activation of myofibroblasts or their differentiation from fibroblasts is driven by ligands from SPP1$^+$ macrophages. In the human kidney, the number of SPP1$^+$ macrophages correlates with the number of collagen-expressing cells[178]. The same population of macrophages has also been identified in the liver, and SPP1$^+$ macrophages expand in cirrhosis and are particularly present in areas of fibrotic liver[177]. The source of SPP1$^+$ macrophages is probably monocytes, as suggested by trajectory analyses in liver cirrhosis[177] and murine heart fibrosis after myocardial infarction[178]. It has recently been demonstrated that human monocytes can be differentiated towards SPP1$^+$ macrophages using a growth factor–cytokine cocktail (GM-CSF, IL-17A and TGFβ1) in vitro[179]. Importantly, these SPP1$^+$ macrophages were able to induce subsequent activation of fibroblastic cells[179].

It is becoming increasingly accepted that part of the miscommunication between macrophages and fibroblastic cells in fibrosis is contact mediated. In vitro studies suggest that fibroblast contractions attract macrophages into their vicinity by displacements in the fibrous collagen ECM, analogous to how flies (fibroblast) trapped in a spider net (collagen) attract the spider (macrophage)[180]. Once within reaching distance, the two cell types can establish physical contact, such as through heterocellular cadherin 11 adherence junctions. Both fibroblasts and macrophages in their activated profibrotic states upregulate cadherin 11, which allows for homotypic binding in vitro and in fibrotic mouse and human lung tissues[181]. The contact-established fibrotic niche is rich in active TGFβ1 that locally maintains myofibroblast activation and contraction[181].

When evaluating strategies for therapeutic killing or de-activation of myofibroblasts in fibrosis, it should be re-emphasized that their ECM-producing and remodelling repair activities are crucial to maintaining organ integrity after injury[82]. Importantly, key mechanisms of fibroblast-to-myofibroblast activation seem to be comparable in normal and pathological tissue remodelling and across different fibrotic conditions and affected organs. This commonality opens opportunities for drugs to target multiple organ fibroses, but also bears the risk that normal healing will be equally affected.

Macrophages are also crucially involved in controlling other immune cell populations and regulating immune tolerance mechanisms, and they have a crucial role in tissue repair and resolution of fibrosis. However, discussion of these roles is beyond the scope of this article, and we refer to other specialized reviews[176,182–184]. It is also important to note that fibroblasts are increasingly recognized as immune sentinel cells that regulate macrophages and other immune cells by expressing molecules such as CCL19 and CCL21 (ref. 185), and this crosstalk might be important in prolonging profibrotic activity. The role of fibroblasts in immune cell activation and regulation has been reviewed in detail recently[186].

Although the above-described mechanisms are largely conserved across organs, it is important to discuss organ-selective or organ-predominant disease processes, as the relevance of each biological process might vary between different organs, with implications for the development of organ-specific antifibrotic therapies. In the following sections, we discuss mechanisms of lung, liver, skin, gut and kidney fibrosis. Cardiac fibrosis is not discussed here, and we refer readers to dedicated review articles[187–189].

Pulmonary fibrosis

Definition and specific mechanisms

The term pulmonary fibrosis is typically reserved for scarring disorders that affect the interstitium of the lung (the region bounded on one side by the alveolar epithelium and on the other by the capillary endothelium)[190]. Airway-centred fibrosis occurs in conditions such as asthma and obliterative bronchiolitis, but this is not typically considered to fall under the term pulmonary fibrosis. More than 200 ILDs have been identified and most of these have the potential to cause pulmonary fibrosis. The most frequently encountered are IPF, autoimmune-associated ILD (most commonly seen with rheumatoid disease and scleroderma) and chronic hypersensitivity pneumonitis. These conditions typically occur in adults aged 60 or over and present with breathlessness that frequently progresses to respiratory failure and death. The most common of these conditions, IPF, affects more than 150,000 people in the USA alone, and has an untreated median survival of 3–3.5 years[191].

The pathogenesis of pulmonary fibrosis has been best described in the context of IPF (Fig. 3). The disease arises in genetically susceptible individuals with polymorphisms in genes including those related to telomere function (such as *TERT* or *TERC*)[192], cell division (such as *TP53* or *CDKN1A*)[193] and host defence (such as *MUC5B*)[18], which have also been identified as risk factors for the development of pulmonary fibrosis in individuals with hypersensitivity pneumonitis and rheumatoid-associated ILD[194,195]. Notably, with regard to the general importance of epithelial injury as an initiator in fibrosis, these polymorphisms appear to render alveolar epithelial cells susceptible to early senescence, which is accelerated by environmental exposure to inhaled injurious agents such as cigarette smoke and wood, metal or stone dust[196]. Senescence of alveolar epithelial stem cells is important because it ultimately causes a failure of re-epithelialization of the alveolar basement membrane following injury in mice[197]. Denudation of the basement membrane stimulates pathways involved in the normal wound healing response and activation as well as transformation of fibroblasts into myofibroblasts. Additionally, recruited monocyte-derived alveolar macrophages contribute to the profibrotic milieu by synthesizing and locally secreting profibrotic growth factors in mouse models[198]. The collagens and ECM proteins secreted by activated fibroblasts and myofibroblasts result in changes in the composition and stiffness of the lung parenchyma, which in turn acts as a further profibrotic stimulus[199] (Fig. 3).

Although the initiating factors for other forms of ILD differ, the pathological mechanisms that drive the development of pulmonary fibrosis in other ILDs, including SSc-ILD, appear to be shared with IPF. This includes the development of similar epithelial phenotypes with abnormal accumulation of intermediate aberrant epithelial cells, and activation of pathways known to regulate AEC2 cell differentiation, accumulation of activated fibroblasts and myofibroblasts and the involvement of profibrotic macrophages[200,201]. These similarities should permit the therapeutic targeting of fibrotic mechanisms across individual ILDs.

Pulmonary fibrosis is characterized by alterations in the pulmonary vasculature with loss of capillaries within regions of fibrosis[202]. The vascular endothelium and vascular smooth muscle cells demonstrate an activated phenotype in pulmonary fibrosis in part due to loss of bone morphogenic protein receptor 2 (BMPR2) expression[203]. Activated endothelial cells in turn act as a stimulus for fibrotic myofibroblasts. These changes are associated with loss of endothelial barrier function that favours recruitment of circulating immune cells[204]. The vascular

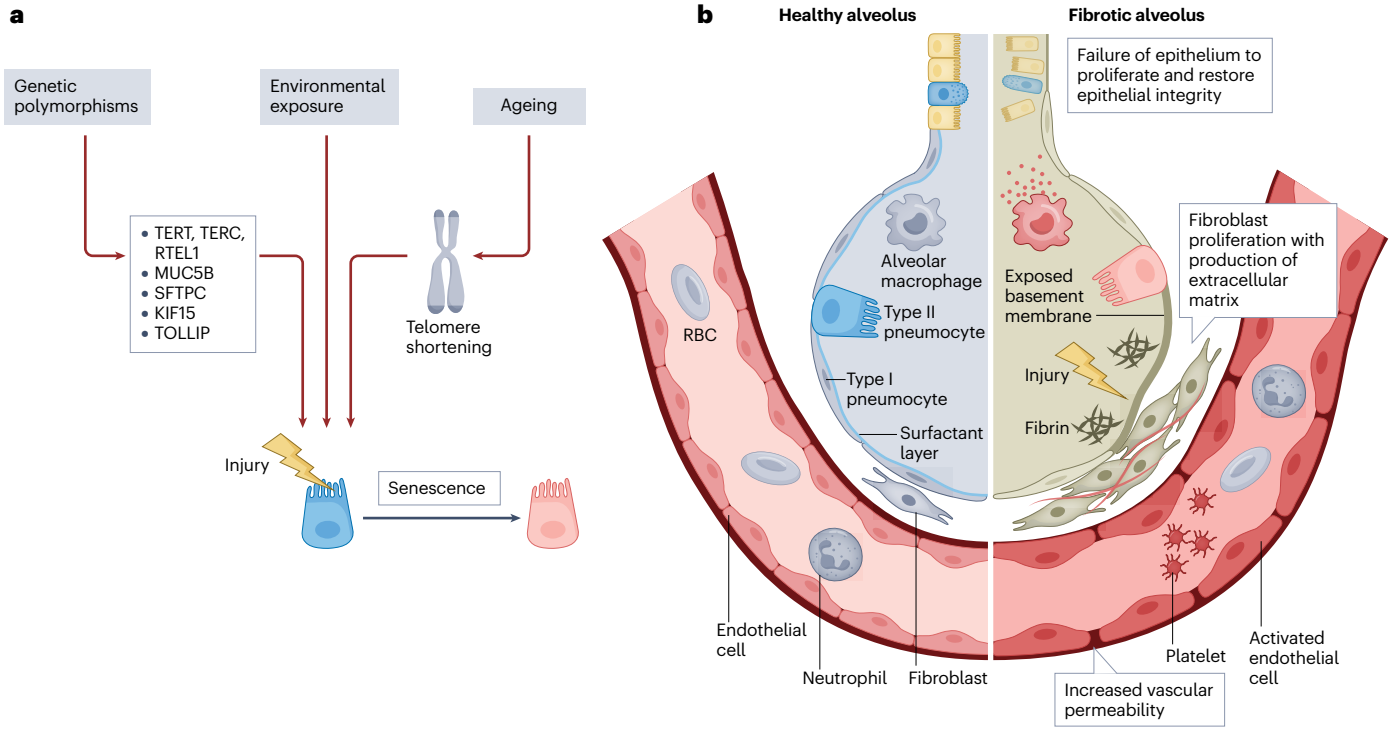

Fig. 3 | Key mechanisms involved in the development of pulmonary fibrosis. **a**, Premature senescence of the type 2 alveolar epithelial cell. This is driven by a combination of genetic polymorphisms, environmental exposure (inhaled dust, viruses and cigarette smoke) and/or autoimmune disease that results in cell injury and ageing with telomere shortening and attrition. **b**, This premature senescence of type 2 cells results in a failure of re-epithelialization of the basement membrane following subsequent injury. This in turn leads to activation of fibroblasts with transformation into myofibroblasts, which then produce collagen and extracellular matrix. Endothelial cell activation results in vascular leak and activation of platelets, fibrin and other components of the clotting cascade. Resident alveolar macrophages and circulating monocytes contribute to the profibrotic milieu by producing profibrotic growth factors. These changes lead to impaired gas transfer and ultimately to respiratory failure. MUC5B, mucin 5B; RBC, red blood cell.

changes seen in pulmonary fibrosis drive the development of pulmonary hypertension, which contributes to morbidity and mortality. Treatment of pulmonary hypertension with drugs such as treprostinil slows progression of fibrosis[205].

Several animal models recapitulate, to some extent, the biological processes observed in pulmonary fibrosis (Supplementary Table 1). The mechanisms to initiate fibrosis in these models include bleomycin, adenoviral TGFβ overexpression, radiation and fluorescein isothiocyanate (FITC)[206]. Of these, the bleomycin model is the most widely used. In this model, the intra-tracheal instillation of bleomycin results in widespread alveolar epithelial injury, which triggers an initial inflammatory response and then later a fibrotic response. The fibrosis is followed by a partial resolution and/or remodelling phase after about 28 days. Although an imperfect model of IPF, the bleomycin murine model has the greatest translational relevance when putative therapies are dosed therapeutically; that is, 7–10 days after bleomycin administration during the fibroproliferative phase of the model[206].

Whereas at least partial resolution of pulmonary fibrosis is seen in animal models, reversal of pulmonary fibrosis in humans has not been observed. By the time individuals present with clinically evident disease, there has often been loss of more than 50% of the gas exchange surface area of the lung. At the microscopic level, this is characterized by loss of terminal airways, destruction of alveolar capillary units and remodelling of the lung with the development of honeycomb cysts lined by hyperplastic epithelium[207,208]. The loss of the fine 3D ultrastructure of the alveolar capillary units in what is an air-filled organ makes it difficult to envisage true reversal of established pulmonary fibrosis, and it is only likely to be feasible in the earliest stages of disease before loss of lung architecture has occurred.

Assessment in clinical practice and clinical trials

Historically, the diagnosis of specific forms of pulmonary fibrosis (Table 1) relied on surgical lung biopsy. The recognition that different histopathological entities have distinct radiological appearances has resulted in computed tomography (CT) forming the cornerstone of diagnosis in most patients with ILD. The gold standard for confirming an ILD diagnosis is the multidisciplinary team (MDT) conference whereby clinicians come together with radiologists and, where necessary, pathologists, to integrate clinical history with other findings[209]. In late-phase clinical trials in IPF, this MDT approach is partially recapitulated through centralized assessment of CT images and biopsy specimens. This has become an important mechanism to ensure the recruitment of a homogeneous population of patients with predictable 12-month disease behaviour. Although CT interpretation is still reliant on radiologists, major efforts are being made into the development of automated computerized tools to facilitate more reliable diagnostic

assessment of CT imaging and to enable better disease quantification and assessment of progression of fibrosis across serial scans[210].

The other important assessment performed in patients with ILD is pulmonary function testing. Although this is less important in making a diagnosis, physiological assessment is invaluable to determine disease severity and to monitor disease progression. At baseline, both forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DL_{CO}) are predictive of prognosis[211]. At follow-up, FVC trajectory is an important measure of disease behaviour, prognosis and response to treatment[212] (Table 1). The importance of FVC decline in pulmonary fibrosis as a surrogate for survival has been acknowledged by the FDA and other regulatory agencies, and for this reason FVC is now the accepted (and expected) primary end point for late-phase pulmonary fibrosis trials[213] (Table 2).

Centralized assessment of CT scans and the use of FVC (measured with standardized spirometers and overseen with central over-reading) as the primary end point were pivotal in the approval of nintedanib and pirfenidone for the treatment of IPF (and the subsequent approval of nintedanib for scleroderma-associated-ILD and progressive pulmonary fibrosis in 2019) (Table 2). Despite these successes, a need remains for clinical trial end points to complement and even supplant FVC[214]. Ideally, new end points would allow CT end points earlier than the current standard of 12 months, which may be accomplished by future artificial intelligence (AI)-based algorithms[215]. Although several commercial systems are available and have been used as exploratory end points in early-phase IPF trials, none has yet been prospectively validated.

In addition to CT, multiple blood-based proteins have been identified that associate with disease severity and progression of pulmonary fibrosis. These include matrix metalloproteinase 7 (MMP7), CCL18, CA125, CA19-9, CYFRA21-1, sICAM1 and collagen synthesis and degradation fragments (CRPM, C3M, pro-C3 and pro-C6)[216–220]. Furthermore, several of these markers, including CA125, sICAM1 and C3M, have been shown to respond to treatment with nintedanib[221]. Further validation is, however, required to better understand the relationship between blood biomarker change, FVC change and survival before these measures can be used in late-phase trials (Table 2).

The antifibrotic small-molecule drugs pirfenidone and nintedanib were approved as therapies for IPF in 2014. Pirfenidone targets myocardin-related transcription factor signalling, which is activated in myofibroblasts that accumulate in the lungs of patients with IPF[222], and nintedanib inhibits PDGF, FGF and VEGF receptors, which are involved in fibroblast proliferation, migration and differentiation, and the secretion of ECM[223]. These approvals provided further stimulus to an already active therapeutic area (Table 3). Although various therapeutic strategies have failed in late-phase IPF trials since 2014, including those targeting IL-13, autotaxin, galectin 3, CTGF and pentraxin[224,225], promising phase II data for several drugs have recently been obtained and they are entering phase III trials. As befits a disease with a complex pathogenesis, these late-phase drugs — nerandomilast, admilparant, bexotegrast and treprostinil[205,226–228] — target different mechanisms. Nerandomilast, a phosphodiesterase 4B (PDE4B) inhibitor, and bexotegrast, a dual inhibitor of the integrins avβ6 and avβ1, inhibit fibroblast proliferation and myofibroblast differentiation, and nerandomilast also has anti-inflammatory activity[226,227,229]. Admilparant inhibits the LPA1 receptor, a key inflammatory mediator that contributes to myofibroblast activation[228], and the prostacyclin analogue treprostinil exerts an antifibrotic effect through activation of the prostaglandin E receptor 2 (EP2), the prostaglandin D receptor 1 (DP1) and peroxisome proliferator-activated receptors (PPARs)[230].

Intestinal fibrosis

Definition and specific mechanisms

IBD, including ulcerative colitis and Crohn's disease, is a relapsing inflammatory condition of the gastrointestinal tract. Intestinal fibrosis is a serious and common complication of IBD, resulting in formation of strictures, obstruction and a need for surgical intervention in more than half of patients with Crohn's disease[231], and motility abnormalities, anorectal dysfunction, rectal urgency and incontinence in patients with ulcerative colitis[232].

Despite identification of more than 200 genes linked with susceptibility to IBD[233], only a few variants have been associated with the development of Crohn's disease strictures. These include genes involved in bacterial sensing, such as the intracellular nucleotide binding oligomerization domain 2 (*NOD2*)[234], although it is unclear whether *NOD2* variants are linked to strictures specifically or to other factors that predispose to stricturing Crohn's disease.

Several mechanisms of fibrogenesis that are somewhat unique to the intestine have been explored and may be targetable. First, inflammation is believed to be an initiator and perpetuator of intestinal fibrosis[231]: the degree of inflammation correlates with the degree of fibrosis in mice and humans, and no report exists in which fibrosis is described to be present outside of areas affected by inflammation[235,236]. In addition to the central fibrosis pathway mediators outlined above, several other cytokines known to be elevated in IBD, such as IL-11 (refs. 237,238), IL-33 (refs. 34,239), IL-34 (ref. 240), IL-36 (ref. 241) and TL1A[242,243], have been implicated in intestinal fibrosis in vitro, in mice and in human samples. A more detailed discussion on the roles of adaptive versus innate immunity can be found elsewhere[244]. Second, gut stromal cells are exposed to, and can sense and respond to, a wide range of pathogen-associated molecular patterns (PAMPs)[239,245]. Inhibition of microbial sensing ameliorates intestinal fibrosis in vitro and in vivo[245]. Third, fat wrapping, so-called 'creeping fat', a pathologically altered mesenteric fat tissue adjacent to Crohn's disease-involved intestinal segments, is strongly associated with Crohn's disease strictures[246,247]. Creeping fat is linked to intestinal smooth muscle thickening, which is a major contributor to luminal narrowing[248] and hence obstruction. Lastly, a recent effort to generate the first full-thickness scRNAseq atlas from stricturing disease and controls revealed that most cell-type and transcriptional changes occur in the mucosa and submucosa over the muscularis propria. Although various cell types appear to have a role as signal receivers, only stromal cells emerged as major signal-sending hubs in stricturing disease. $CXCL14^+$ and HHP^+ fibroblasts, which were over-represented in stricturing disease, are targetable via cadherin 11 (ref. 249) (Fig. 4). Data on the impact of the vasculature on intestinal fibrosis are restricted to a description of the phenomenon of EndMT[250].

Although no animal model completely recapitulates the human situation, multiple preclinical models for intestinal fibrosis have been described[231], allowing translation of the discovered mechanisms. They can be categorized into small bowel and colonic disease location, as well as by their inciting mechanism, into spontaneous models, genetically altered models, chemically induced models or bacteria-induced models[231] (Supplementary Table 1).

Clinical observations suggest that intestinal fibrogenesis is not unidirectional, but reversible. In large observational cohort studies, recurrence at the site of a small bowel strictureplasty was minimal (only 3%)[251], and narrowing of the intestinal lumen was found in only

11% after 2 years[252]. These observations have been confirmed in serial intestinal ultrasound (IUS) examinations[253]. Several mechanisms may be responsible for reversing fibrosis on a molecular level and these are discussed elsewhere[254].

Intestinal fibrosis assessment in clinical practice and in clinical trials

Endoscopic mucosal biopsies are able to sample only the superficial layers of the intestine, and do not reach the submucosa, the gut wall

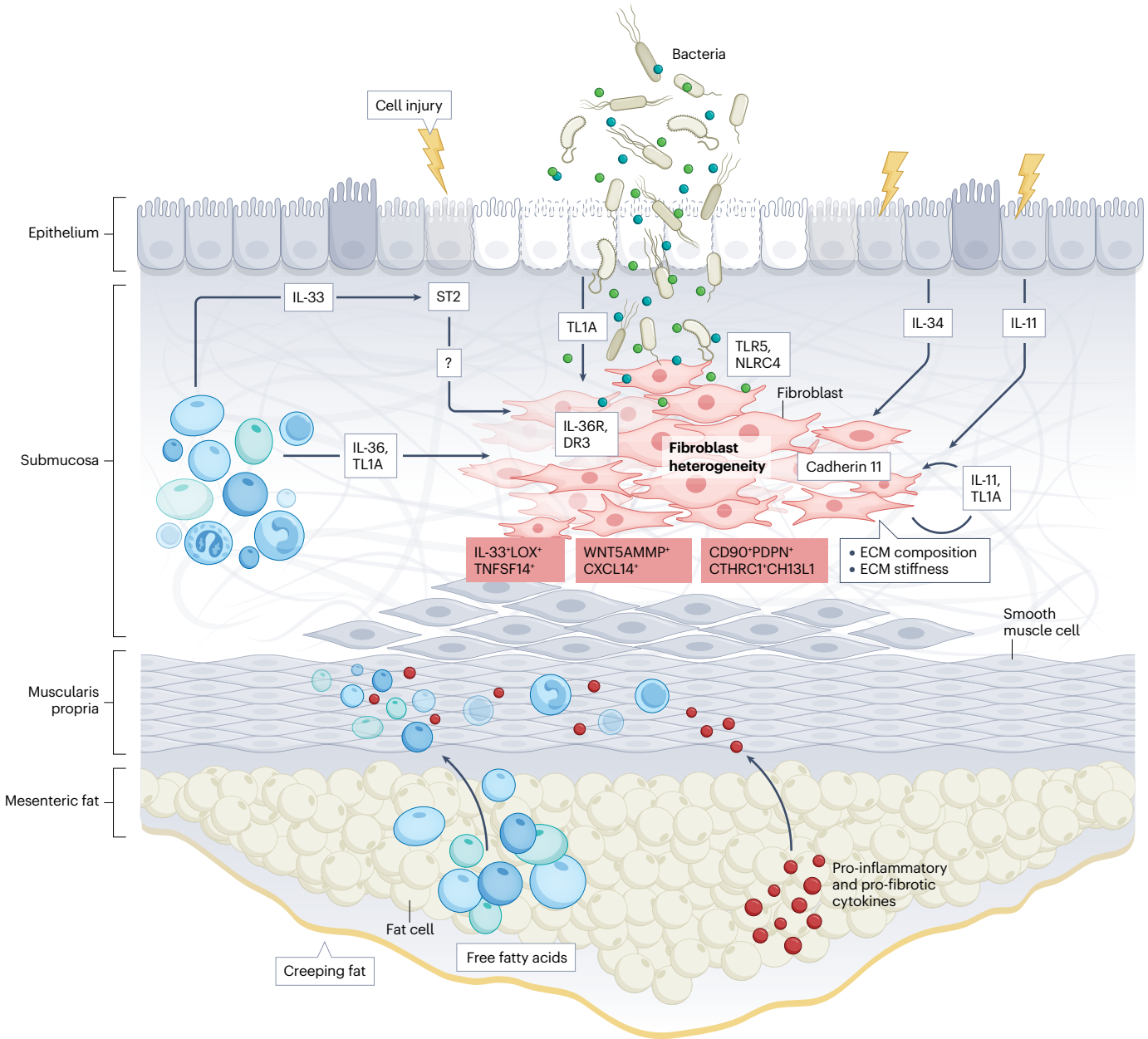

Fig. 4 | Core mechanisms of intestinal fibrogenesis. Mesenchymal cells in intestinal fibrosis are highly heterogeneous and are exposed to direct contact with intestinal immune and non-immune cells, their soluble mediators, and microbial components and metabolites. A 'leaky' epithelial barrier owing to cellular injury permits contact of mesenchymal cells with the microbiota and their products. Those factors can directly or indirectly activate mesenchymal cells through, for example, Toll-like receptors (TLRs) or inflammasome signalling. Activated epithelial cells and immune cells are able to produce profibrotic mediators, such as IL-11, IL-33, IL-34, IL-36 and tumour necrosis factor superfamily protein TNF-like 1A (TL1A). Fibroblasts can signal in a homotypic fashion through cadherin 11, IL-11 or TL1A. The cellular environment provides biochemical and mechanical cues to mesenchymal cells independently of intestinal inflammation. This complex environment gives rise to heterogeneous fibroblast populations linked to fibrostenosis, such as IL-33–lysyl oxidase–tumour necrosis factor superfamily member 14-positive (IL33^{+}LOX^{+}TNFSF14^{+}), Wingless related integration site–matrix metalloproteinase–CXCL14-positive (WNT5A^{+}MMP^{+}CXCL14^{+}) or CD90–podoplanin–chitinase triple helix repeat-containing 1–chitinase 3-like 1-positive (CD90^{+}PDPN^{+}CTHRC1^{+}CH13L1^{+}) cells. Creeping fat can release pro-inflammatory mediators and free fatty acids that act on intestinal muscularis propria smooth muscle cells and fibroblasts, leading to thickening of the muscularis propria. ECM, extracellular matrix.

layer mostly affected by fibrosis[248]. Endoscopic evaluation alone can detect stenosis, but cannot assess the degree of fibrosis[255]. Hence, cross-sectional imaging is the mainstay of stricture diagnosis in Crohn's disease[256]. The accuracy to detect stenosis is high for conventional magnetic resonance enterography (MRE), computed tomography enterography (CTE) and IUS, which is the case despite heterogeneous definitions for strictures[256] (Table 2). To overcome this limitation and to facilitate translation of novel antifibrotics to the field of stricturing IBD, reliable definitions are needed. The global Stenosis Therapy and Anti-Fibrotic Research (STAR) consortium created clear definitions for what constitutes a stricture and its improvement[255]. An ongoing programme is building trial end points and monitoring tools for clinical studies in fibrostenosing IBD, including a patient-reported outcome tool and stricture radiology indices[257,258].

Despite progress in definitions, routine cross-sectional imaging has low accuracy for the quantification of fibrosis within a stricture, which precludes its use in clinical practice or clinical trials[256]. Experimental techniques such as delayed enhancement MRE[259], elastography[260], magnetization transfer[261] or diffusion-weighted imaging[262] have been tested but, to date, none of these approaches could be externally validated. These techniques were able to distinguish only extremes of fibrosis grade (none or mild versus moderate or severe), which is clinically of limited value.

So far, one trial testing a drug with a stricture-targeted mechanism (a TGFβ1 type 1 receptor (activin receptor-like kinase 5 (ALK5)) kinase inhibitor known as AGMB-129; NCT05843578) has started in IBD (Table 3). The predominant reason for this low number has been the lack of consensus on definitions and the lack of clinical end points, both of which are now addressed by STAR (Table 2). There is still a need for clarity on the regulatory pathway for approval of an antifibrotic drug for IBD. Using biomarkers to stratify patients into 'at risk' populations for antifibrotic treatment is a desirable strategy. Multiple biomarkers taken at Crohn's disease diagnosis have been proposed to determine risk for future strictures, but, although academically interesting, none of them is accurate enough to be used in clinical practice or clinical trials[234]. The same is true for biomarkers to quantify fibrosis within an existing stricture, and at this time an end point could not rely on a surrogate marker for fibrosis.

For this reason, the phase II trial of AGMB-129 started with a population with clinically symptomatic established strictures, based on the premise that resection studies in this population clearly delineated the co-existence of inflammation and fibrosis[235]. A lead-in or co-treatment with anti-inflammatory drugs can mitigate obstructive symptoms[255] and allow randomization to antifibrotic drug versus placebo. Absence of obstructive symptoms, stricture morphology changes on cross-sectional imaging or reduced number of endoscopic or surgical interventions may serve as clinically meaningful end points. Ultimately, each end point would need to show that improvement of the end point translates into clinically meaningful improvement for the patient (Table 2).

Finally, to successfully develop markers to predict or quantify fibrosis, development of a histopathological gold standard for full-thickness resection is necessary. Typically, candidate markers are tested in populations with symptomatic strictures, who undergo imaging, followed by bowel resection with grading of the intestinal tissue for inflammation and fibrosis[263,264]. After determination of construct validity by correlation of histological evaluations of the degree of fibrosis and/or inflammation on the resected tissue to imaging results, the novel imaging test is evaluated. Strikingly, no full-thickness histopathology index has been validated following modern methodological standards[265]. Existing scores are highly heterogeneous[266]. One solution would be a reliable, validated and responsive histopathological standard with discriminatory ability to be able to optimize and compare cross-sectional imaging techniques. This work is ongoing in the STAR consortium.

Kidney fibrosis

Definition and specific mechanisms

Kidney fibrosis is the common final stage of nearly all CKDs, which are characterized by interstitial expansion through accumulation of proliferating, ECM-producing, myofibroblasts. The severity of interstitial kidney fibrosis correlates inversely with kidney function as measured by glomerular filtration rate (GFR)[267].

There are genetic diseases that cause kidney fibrosis, such as adult polycystic kidney disease, but also many other known mutations in genes encoding podocytes or glomerular proteins, including *APOL1*, *WT1*, *NPHS1* and *COL4A3*. Genetic association studies also suggest a role of polymorphisms in genes encoding inflammatory cytokines, the renin–angiotensin–aldosterone system, DNA repair, TGFβ and ROS signalling, endocytosis, autophagy, apoptosis and WNT signalling, among others[268–270].

One major conserved mechanism across all kidney diseases is tubule epithelial injury (Fig. 5), again highlighting the cross-organ relevance of epithelial injury as an initiating factor in fibrosis. It can occur owing to leakiness of the glomerular filter with subsequent proteinuria (for example, caused by diabetes or genetic mutations such as those noted above), by direct toxic mechanisms (for example, drugs) or hypoxic injury. Injured tubule epithelial cells dedifferentiate, flatten and acquire expression of injury markers such as KIM1 and mesenchymal markers such as vimentin. This process has been termed partial EMT. Recent studies in mice and human tissues indicate that injured and dedifferentiated proximal tubule epithelial cells have a major role in kidney injury and fibrosis. These cells directly activate pericytes and myofibroblasts to become myofibroblasts by epithelial–mesenchymal crosstalk, but also activate immune cells, which then drive fibroblasts and pericyte activation[55,271]. Most kidney myofibroblasts in mice originate from resident fibroblasts and pericytes[54,271], and other cell types such as endothelial cells, epithelial cells or immune cells do not contribute to the myofibroblast pool[54,271]. The main cellular crosstalk partners of kidney-fibrosis-driving mesenchymal cells are immune cells such as macrophages and injured epithelial cells such as proximal tubule epithelial cells[271] (Fig. 5). Multiple animal models of kidney fibrosis have been proposed to facilitate translation of novel therapies, but each one of them can only partially reflect the human pathophysiology (Supplementary Table 1).

The kidney vasculature has a pivotal role in fibrosis and links fibrosis to kidney functional decline. Perivascular fibroblasts and pericytes get activated and detach from capillaries to become interstitial myofibroblasts[66,272,273]. This leaves the kidney vasculature unprotected and dysfunctional, which leads to capillary loss, driving proximal tubule hypoxic injury. This contributes to inflammation and subsequently more fibrosis[274,275]. Various groups have demonstrated that after kidney injury, and independently of the type of injury, the kidney reacts with a stereotypical response that includes capillary loss and vascular dysfunction[274,276] followed by fibrosis.

Regression of kidney fibrosis, more specifically glomerulosclerosis, has been elegantly demonstrated by the work of Fioretto et al.[277], using renal biopsies taken before pancreas transplantation and 5 and 10 years thereafter presenting mild to advanced lesions of diabetic

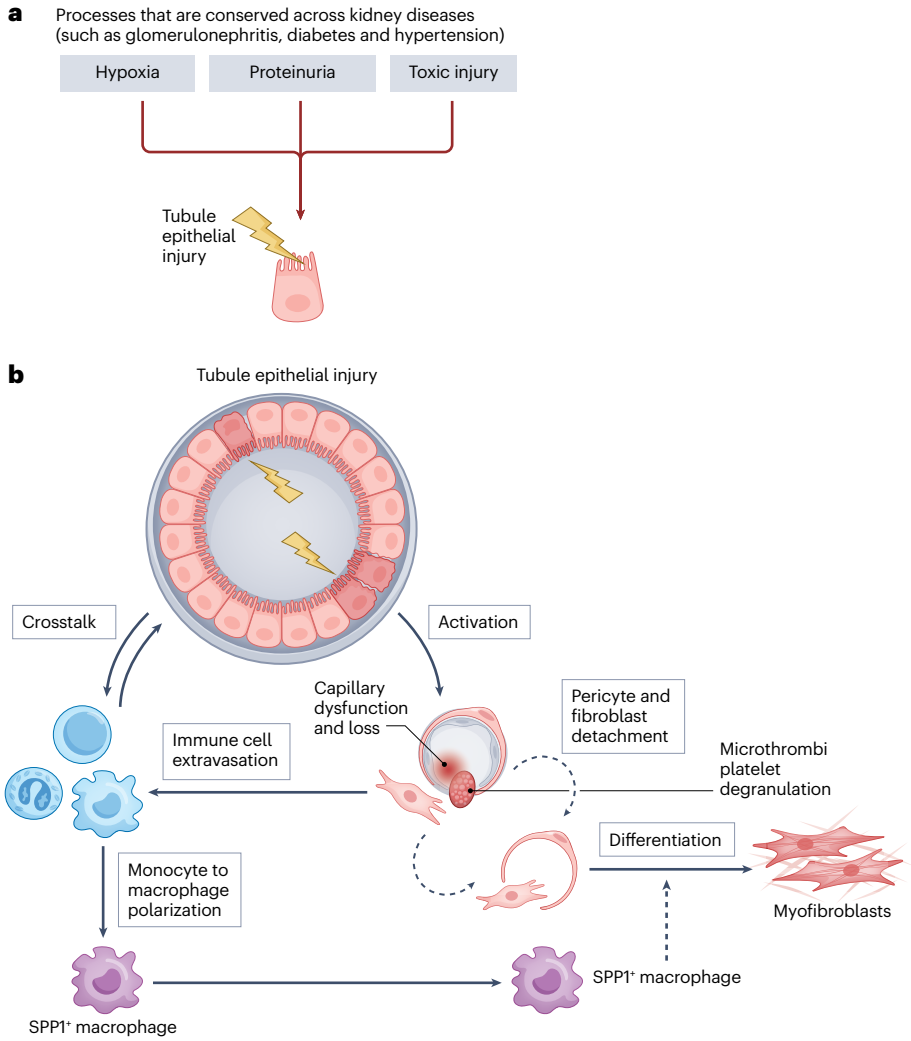

Fig. 5 | Key pathways of chronic kidney disease and fibrosis. a, Tubule epithelial injury is a conserved mechanism across most kidney diseases and can occur as a result of hypoxic injury (for example, in acute kidney injury) or glomerular disease with subsequent proteinuria or owing to toxic injury (for example, due to drugs). **b**, Injured tubule epithelial cells dedifferentiate and drive activation of fibroblasts and pericytes, which detach from capillaries, driving capillary dysfunction, loss and immune cell extravasation. Signalling from epithelial cells and degranulated platelets from microthrombi within the dysfunctional capillaries drives monocyte to SPP1$^+$ macrophage polarization. SPP1$^+$ macrophages (also termed scar-associated macrophages) interact with fibroblasts and pericytes and trigger their myofibroblast differentiation with subsequent extracellular matrix deposition and fibrosis.

nephropathy at the time of transplantation. Although limited to a very small patient subset ($n = 8$), this evidence suggests that reversal of established fibrosis might occur when the causative insult is removed. Unfortunately, the long-standing use of drugs such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers has not been shown to result in similar effects in humans to date, although delayed treatment with an ACE inhibitor led to regression of pre-existing glomerular, tubular and vascular lesions in preclinical models[278].

The regenerative capacity of the kidney is relatively low in comparison with other organs such as the liver and the intestine. In addition, as nephrons cannot regenerate much, it is questionable how much reversal of fibrosis in the kidney would also improve kidney function. Thus, the aim of an antifibrotic strategy would most likely be to stop or slow down kidney fibrosis progression and thereby reduce the ongoing destruction of functional kidney parenchyma and GFR decline.

Kidney fibrosis assessment in clinical practice and in clinical trials

The current gold standard method to measure kidney fibrosis (Table 1) is transcutaneous needle biopsy with subsequent histopathological evaluation and quantification of fibrosis using Sirius red or Masson's trichrome staining or immunostaining of collagen type I. Alternatively, the renal pathologist often estimates fibrosis using the so-called interstitial fibrosis/tubular atrophy score (IF/TA), which lacks reliability between pathologists. However, fibrosis in the kidney might be patchy and thus be misrepresented by the small biopsy sample. A challenge for development of non-invasive imaging tools is the kidney eliminating the contrast agent from the body. Diffusion-weighted MRI (DW-MRI) has been used to quantify kidney fibrosis[279]; however, larger clinical trials using this method are lacking.

No specific antifibrotic therapies for the kidney have been approved to date (Table 3). A single ongoing phase II trial in CKD is testing pirfenidone (NCT04258397), with the primary end point of change from baseline in kidney fibrosis as assessed by DW-MRI and by urinary markers of tubulo-interstitial fibrosis (α1M, PIIINP, MCP1). The secondary end point is the change from baseline in kidney function, as assessed by estimated GFR and urine albumin–creatinine ratio. A previous open label non-controlled trial with pirfenidone in patients with biopsy-proven focal segmental glomerulosclerosis showed an improvement in the rate of decline at 12 months[280]. However, in eight patients who continued

pirfenidone treatment for an additional 24–48 months, the significant improvement in GFR decline rate seen in the first 12 months was not maintained. Pirfenidone has also been tested in a small diabetic nephropathy trial showing an effect on GFR loss at a dosage of 1,200 mg per day, whereas a dosage of 2,400 mg per day did not show an effect[281]. Direct inhibition of TGFβ signalling using an anti-TGFβ antibody has also been tested in patients with focal segmental glomerulosclerosis and diabetic nephropathy, without significant effect[282,283].

A major issue in the development of renal antifibrotic therapies is lack of optimal end points (Table 2). Large patient groups and long follow-up times are needed to assess renal function, which makes trials too costly. For a drug for which the primary mechanism of action is in the glomerulus, quantification of proteinuria (usually as albumin–creatinine ratio) allows an early end point; however, for therapeutics that target interstitial kidney fibrosis, measurement of proteinuria is less useful. As mentioned above, non-invasive imaging technologies such as DWI-MRI or other imaging technologies that also contain probes (for example, for elastin[284]) are currently being tested and could potentially be transformational for this disease area.

Skin fibrosis

Definition and specific mechanisms

Dermal fibrosis is the main consequence of skin diseases such as hypertrophic scars, keloids or morphea (localized scleroderma), but can also be the leading manifestation of systemic fibrosing diseases such as SSc[285], which we focus on here. For other fibrosing skin diseases, we refer to recent reviews[286,287].

SSc affects the skin as well as the lungs, the heart and the intestine. Skin fibrosis starts at the distal extremities and progresses proximally. SSc-associated ILD often manifests within the first 5 years after diagnosis and is often progressive. Cardiac fibrosis can occur throughout the disease, whereas intestinal fibrosis is mainly observed in later stages of the disease.

The broad spectrum of clinical manifestations of SSc leads to severe morbidity and mortality. Indeed, despite recent improvements in the prognosis of SSc, it remains the autoimmune rheumatic disease with the highest case-specific morbidity, with approximately half of the patients dying as a direct consequence of the disease[288].

SNPs are associated with the risk of SSc and the risk to develop certain organ complications. The identified SNPs predominantly affect genes associated with immune processes, but not fibroblasts, endothelial cells or epithelial cells, and are often shared with other autoimmune diseases such as systemic lupus erythematosus (SLE)[289]. Annotated loci associated with the SSc trait are summarized in Supplementary Table 3 (ref. 290). Their discovery has contributed tremendously to our understanding of SSc pathogenesis. However, the odds ratio for all of these SNPs is modest and the genetic component in this disease is thought to be minor[289].

Histopathologically, SSc is characterized by microangiopathy, inflammation, autoimmunity and fibrosis[40]. Apoptosis of endothelial cells in a genetically susceptible individual in response to yet-unidentified triggers is thought to be the first manifestation of SSc[39], promoting perivascular injury and autoimmunity, which subsequently triggers fibroblast activation and tissue fibrosis[291] (Fig. 6).

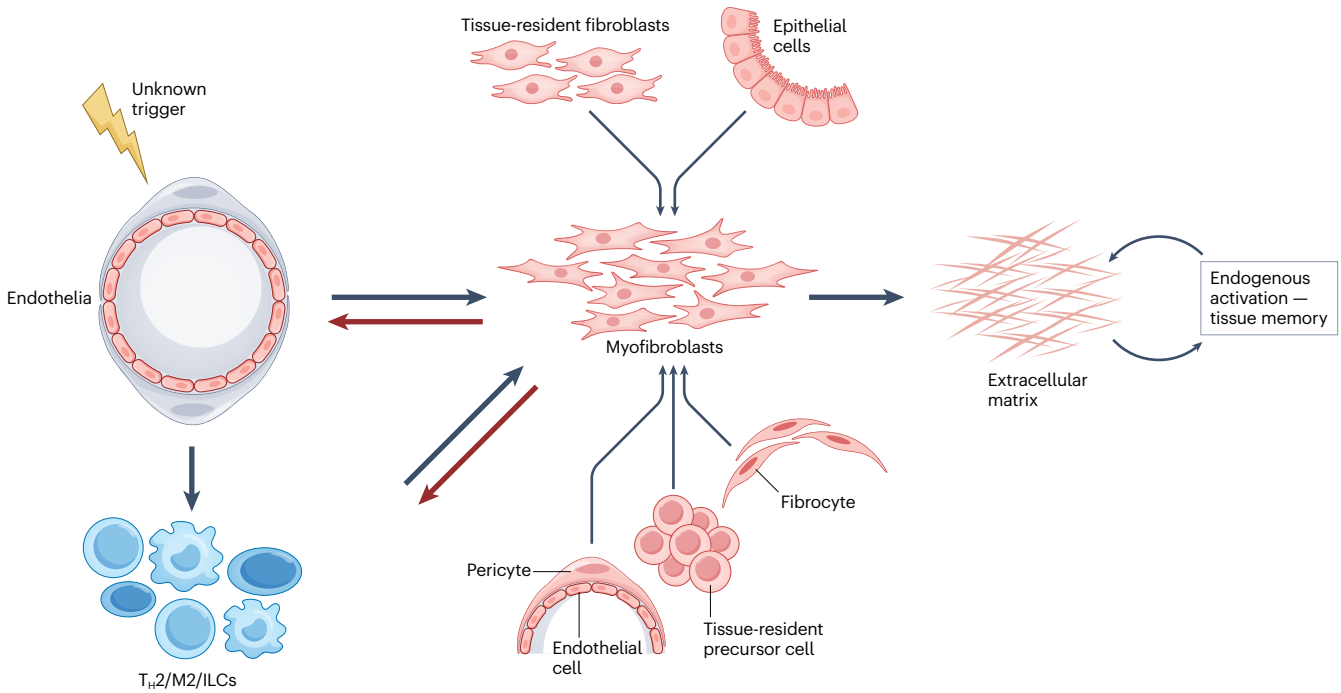

Fig. 6 | Mechanisms of fibrotic tissue remodelling in systemic sclerosis. As-yet-unidentified triggers promote apoptosis of microvascular endothelial cells as the first histopathological change in systemic sclerosis (SSc). Microvascular injury promotes leukocyte infiltration and type 2 immune responses, with release of profibrotic mediators from alternatively activated (M2) macrophages, T helper 2 (T_H2) cells and innate lymphoid cells (ILCs). Injured endothelial cells as well as activated platelets also release profibrotic factors. The resulting profibrotic milieu activates resident fibroblasts and induces transdifferentiation of various cell types to myofibroblasts. Myofibroblasts in turn release soluble mediators that may aggravate vascular manifestations and modulate inflammation in SSc. Progressive tissue remodelling induces self-sustaining activation loops that maintain fibroblast activation even in the absence of exogenous stimuli.

The early stages of fibrotic disease are characterized by complex inflammatory events that involve both the innate and adaptive immune systems. As for fibrosis in other organs, the inflammatory response is type 2 biased, with release of T helper 2 (T_H2) cytokines such as IL-4 and IL-13 from alternatively activated macrophages, T_H2 cells and innate lymphoid cells type 2 (ILC2s)[40]. Classical clinical or laboratory features of inflammation such as arthritis, myositis or elevated C-reactive protein (CRP) are observed in only approximately one-third of patients in cross-sectional studies. B cells are activated in SSc and are found in increased numbers in fibrotic skin. B cells can drive disease progression through the release of profibrotic cytokines such as TGFβ1 and IL-6 (ref. 292), as well as autoantibodies. Autoantibodies were long thought to be only markers for disease classification[293], but functional, pathogenic autoantibodies to PDGF receptors, to GPCRs such as the endothelin and angiotensin receptors and to undefined epitopes on endothelial cells have now been identified in SSc[294,295]. These autoantibodies might maintain chronic vascular injury and fibroblast activation even after resolution of inflammation.

Following these early events, fibrotic remodelling of the skin is facilitated by progressive deposition of ECM by myofibroblasts (Fig. 6). Various different cell types can acquire myofibroblast features in the skin. Besides resident resting dermal fibroblasts, cells of the vascular wall, such as endothelial cells and pericytes, are thought to be important sources of myofibroblasts in the skin. Moreover, mesenchymal precursor cells, such as adiponectin-positive adipogenic precursor cells in the subcutis, can acquire a myofibroblast phenotype[296]. Transdifferentiation of these adipogenic precursor cells might also account for the progressive atrophy of the subcutis that is seen in most patients with advanced SSc. In contrast to other organs, the basal membrane acts as a tight barrier that hinders transdifferentiation of epidermal keratinocytes as epithelial cells of the skin into myofibroblasts. However, not only the increased differentiation of precursor cells into myofibroblasts but also their impaired removal is thought to drive progression of fibrosis in SSc. In contrast to normal repair processes (for example, after physical wounding), myofibroblasts in SSc are not removed by apoptosis or deactivated but persist and continue to release abundant amounts of ECM. This persistently activated phenotype of myofibroblasts in SSc is maintained by epigenetic imprinting[297]. This so-called tissue memory renders myofibroblasts endogenously active and thus independent of external stimuli; for example, of infiltrating immune cells.

Drug development for SSc focuses on skin and lung fibrosis. Numerous preclinical models have been developed to test antifibrotic effects in skin and lung fibrosis, including mouse models, traditional 2D cell culture assays with primary cells, multicellular, 3D model systems and ex vivo cultures of patient-derived tissues[298,299]. None of the preclinical models encompasses all the clinical and molecular manifestations of SSc. Most mouse models resemble inflammatory and fibrotic features of SSc in certain tissues, but do not cover the full spectrum of organ manifestations (Supplementary Table 1). Moreover, the pathophysiological cascade from microvascular injury and inflammation to fibroblast activation and fibrosis is incompletely reflected.

All mouse models have limitations, and a thoughtful combination of different mouse models is a mandatory part of the preclinical portfolio for regulatory agencies. However, drug candidates for SSc are now also tested using human multicellular organ models such as organ-on-chip models assembled from different types of adult human cell, or organoids, generated from patient-derived induced pluripotent stem cells (iPS cells). Although these models overcome several of the central limitations of traditional 2D cell culture experiments such as derivation from interactions with a physiological 3D ECM and crosstalk with other cell types, they do not encompass all features of diseased SSc tissue (for example, they focus on a few cellular players in organ-on-chip models or rather immature tissue in organoids). Ex vivo culture models such as precision-cut tissue slices include all relevant cell types and maintain all cellular niches in affected tissues but cannot be cultured long term without introduction of cell culture artefacts and are also not suited to study cellular recruitment (for example, of circulating immune cells).

Skin fibrosis assessment in clinical practice and in clinical trials

Skin fibrosis in patients with SSc is commonly assessed by the modified Rodnan Skin Score (mRSS), which involves scoring 17 areas of the body on a scale from 0 to 3 (where 0 is normal skin and 3 severe thickening) and summing up the scores of all areas, leading to total mRSS scores of 0–51 (ref. 300). Although training can help to align scores between different investigators, inter-observer variability remains a limitation. Another point to consider for clinical trials is that the mRSS regresses spontaneously over time, even in untreated patients.

Therapeutic approaches for SSc rely on the following pillars: symptomatic therapy, vasoactive therapy, immunomodulatory therapy (such as with mycophenolate mofetil[301] and cyclophosphamide[302]) and most recently antifibrotic, fibroblast-targeting therapy (Table 3). The anti-IL-6 receptor antibody tocilizumab failed to meet its primary end point on skin fibrosis, but prevented progression of ILD in trials of patients with early, inflammatory SSc[303,304] and has been approved by the FDA for the treatment of SSc-ILD. The multi-tyrosine kinase inhibitor nintedanib, which predominantly targets fibroblast-activating pathways, reduced progression of SSc-ILD, but not of skin fibrosis[139] and is also approved for the treatment of SSc-ILD (Table 3).

These results highlight that inflammation and fibroblast activation both offer potential for targeted intervention in SSc. Initial evidence from the nintedanib trial indicates that combinations of fibroblast-targeting agents with immunomodulatory treatments such as mycophenolate mofetil may have additive effects[305]. Thus, anti-inflammatory and immunomodulatory therapies have a higher significance in SSc than in most other fibrotic disorders and inform current drug development programmes that include anti-inflammatory or immunomodulatory agents as well as fibroblast-targeting compounds.

Evaluation of the efficacy of these treatments currently requires long follow-up. As fully validated biomarkers of fibrotic activity are currently lacking, fibrotic disease activity and thus treatment efficacy cannot be quantified directly, but instead is extrapolated retrospectively from the accrual of damage over time (for example, increase in the mRSS as a marker of skin thickening or loss of FVC owing to fibrotic remodelling of the lungs). Circulating collagen neoepitopes[306], as well as molecular imaging with FAPI-based quantification of activated fibroblasts by positron emission tomography (PET)–CT[307,308], may offer potential as biomarkers of fibrotic activity in SSc but require further validation (Table 2).

Liver fibrosis

Definition and specific mechanisms

Liver fibrosis is associated with viral hepatitis, autoimmune hepatobiliary diseases (including autoimmune hepatitis, primary sclerosing cholangitis and primary biliary cirrhosis) and metabolic liver diseases (metabolic dysfunction-associated steatotic liver disease[309] (MASLD, formerly termed NAFLD/NASH) and alcohol-associated liver

disease). With the reduction in the number of patients affected by virus-associated liver diseases such as hepatitis C owing to the use of direct-acting antivirals, the rise in fibrosis in patients with metabolic liver disease has now become one of the most critical problems in the hepatology field, with a predicted two- to threefold rise in MASLD incidence worldwide from 2016 to 2030 (ref. 310).

Genetic factors that predispose to liver fibrosis identified to date are primarily polymorphisms in genes involved in the regulation of lipid metabolism in hepatocytes, including *PNPLA3*, *HSD17B13* and *MBOAT7* (ref. 311). This is consistent with the role of epithelial cell/hepatocyte injury as key initiating factors in hepatic fibrosis. There is also a growing appreciation that the fibrotic activity of HSCs can be affected by *PNPLA3* polymorphisms, as well as other genes that directly regulate HSC activation[312–314]. Polymorphisms in genes encoding cytokines such as IL-1β, TNF and IL-17 are also likely to contribute to disease susceptibility[314].

Progression of hepatic fibrosis in response to injury involves complex interactions between multiple cell types in the liver, with strong links between hepatocyte injury, activation of innate immune cells and production of ECM (Fig. 7). Studies in animal and cell culture models have revealed that ECM is produced primarily by activated HSCs in response to hepatocyte injury or periductular fibroblasts with biliary injury. In the healthy liver, HSCs are the primary site for storage of retinyl esters; upon activation in response to injury, the retinyl ester stores are lost and the HSCs develop a myofibroblast-like phenotype. Primary drivers of HSC activation include apoptotic hepatocytes and profibrogenic cytokines generated by both resident hepatic macrophages and infiltrating monocytes[79]. Innate immune cells in the liver are activated by locally produced DAMPs released by injured hepatocytes and PAMPs leaking from the gut into the portal circulation. Other components of the immune system, including complement and adaptive immune cells, in both the liver and the gut, have also been shown in murine models to contribute to hepatic fibrosis[79,315].

The vasculature of the liver is highly complex, including portal and central vasculature, as well as the microvasculature lined by liver sinusoidal endothelial cells (LSECs). The vascular changes during fibrosis occur predominantly in the microcirculation. LSECs can express several matrix proteins, thus directly contributing to fibrosis. LSECs also release profibrogenic cytokines and growth factors that have paracrine effects on HSCs. Importantly, an imbalance between vasoconstrictors and vasodilators, including PDGF, endothelin, TGFβ and nitric oxide, contributes to the development of portal hypertension characteristic of liver fibrosis[316,317].

Much of our understanding of the mechanisms for the progression and regression of liver fibrosis derives from studies in animal models[318,319]. No single model of hepatic fibrosis completely reproduces the human condition, but multiple murine models are currently used to understand pathophysiological mechanisms and interrogate novel drug targets (Supplementary Table 1). Importantly, growing evidence comparing fibrotic responses in patients with various murine models indicates that preclinical models are congruent with many characteristics of the human fibrotic liver[177,320]. However, it is crucial to remember that the strain of mice used for studies has a strong influence on fibrotic responses[321]. Recent studies have also illustrated the utility of

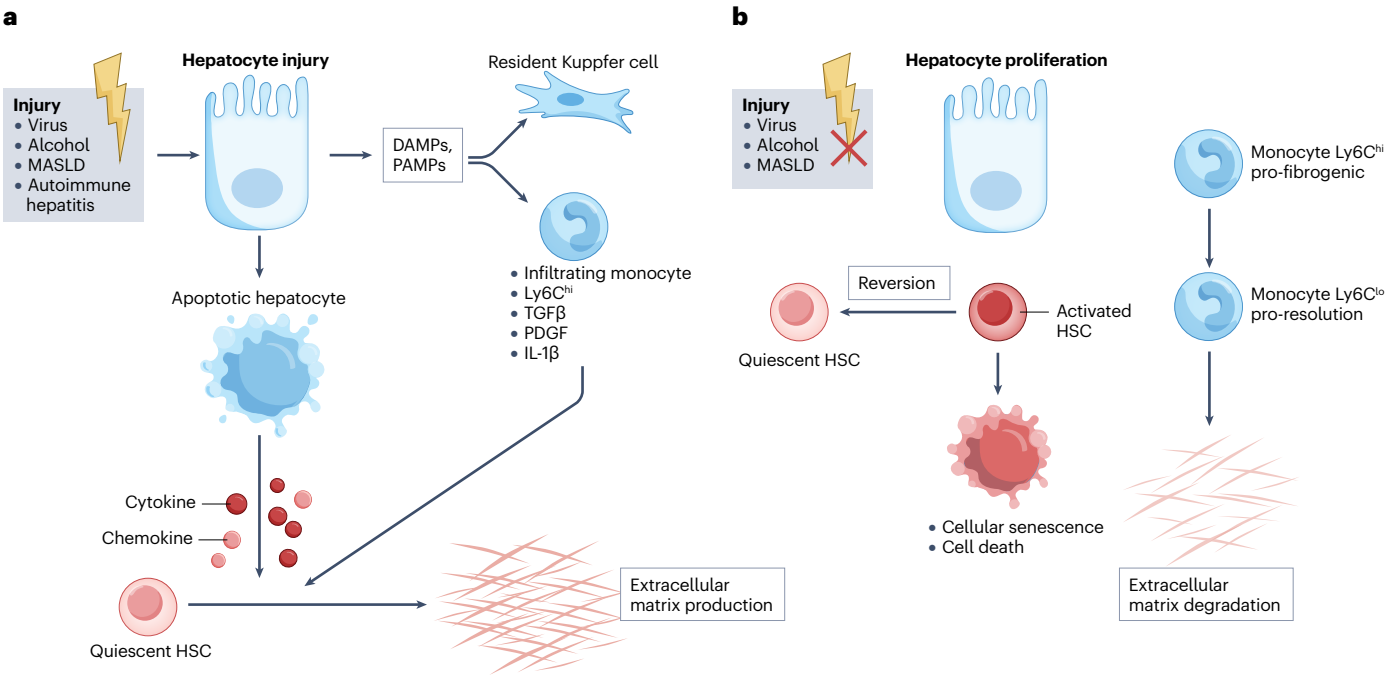

Fig. 7 | Key pathways of liver fibrosis progression and regression. **a**, Injury to hepatocytes results in the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) that activate resident and infiltrating immune cells in the liver and hepatic stellate cells (HSCs). In turn, pro-inflammatory cytokines and chemokines and profibrogenic cytokines are released that further stimulate the differentiation, migration and activity of HSCs. **b**, Regression of fibrosis occurs if the damaging insults are removed. Then, the hepatocytes can proliferate to replace injured hepatocytes, and HSCs are inactivated via reversion to a quiescent phenotype, cell death or cellular senescence. In murine models, extracellular matrix degradation is promoted by the activity of pro-resolution Ly6C^{lo} monocytes and the activity of matrix metalloproteinases. MASLD, metabolic dysfunction-associated steatotic liver disease; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor-β.

using humanized mouse models to overcome the species differences in fibrogenic responses in the liver[322].

Recent advances in the use of in vitro model systems, including hepatic organoids, precision-cut liver slices and liver-on-a-chip models, provide useful platforms to study mechanisms of fibrotic injury and repair. 3D culture systems incorporating multiple cell types (hepatocytes, HSCs and macrophages) derived from human iPS cells hold promise for determination of human-relevant drug targets for fibrosis[318,323]. The use of human iPS cells is particularly important for studies of liver fibrosis to overcome the difficulty of translating work done in murine models to the human condition[318]. Furthermore, there is a growing availability of large omics data sets and biorepositories from both patients with hepatic fibrosis of different aetiologies and population cohorts.

It is generally accepted that hepatic fibrosis can regress once the cause of insult (such as a virus, toxic drug or metabolic disease) is removed. The liver has far greater regenerative capacity than other organs, but it is unclear whether complete regression of hepatic fibrosis is possible, particularly in more advanced stages at which substantial architectural changes have already occurred. Regression may also depend on the type of injury, with biliary fibrosis likely to be more reversible than hepatotoxic injury[324].

At the cellular level, regression of fibrosis depends on multiple cell types. The fate of the activated HSC is crucial; HSCs can revert to a quiescent state, undergo cell death and/or be inactivated via cellular senescence. Immune cells in the liver have an important role in resolution of fibrosis; in murine models, a subpopulation of restorative $Ly6C^{lo}$ monocytes, the murine equivalent of $CD14^{+}$, $CD16^{++}$ monocytes, exhibit active pro-resolution and anti-inflammatory properties[325]. The crucial balance between MMPs and tissue inhibitors of metalloproteinases (TIMPs) regulates the dynamic turnover of ECM.

Hepatic fibrosis assessment in clinical practice and in clinical trials

The reference standard for the assessment of hepatic fibrosis is histological examination through liver biopsy (Table 1). A standardized scoring system allows for accurate staging of fibrotic injury from mild fibrosis (F1–F2) to more severe stages (F3–F4). However, in clinical practice, non-invasive measures of fibrosis are important[326,327]. Biochemical improvement in liver function is an important indicator of fibrosis regression; recent data from the MASH Clinical Research Network indicate that reduction in fibrosis improves liver-related outcomes[328]. Non-invasive simple fibrosis scores, blood biomarkers and imaging are currently used in clinical practice or are in development for clinical trials (Table 2). Current research is beginning to incorporate multi-omics approaches for the generation of sensitive and accurate biomarkers for liver fibrosis[329].

Regression of fibrosis in specific populations is often difficult to assess, as repeated liver biopsies are not carried out and non-invasive biomarkers are not yet approved for use in clinical trials. However, the identification of additional modifiers, such as genetics, in the regression of fibrosis is an active field. Indeed, there is currently no fixed definition for fibrosis regression; future advances in the use of non-invasive markers, in particular, magnetic resonance-based imaging, will be crucial to better understand fibrosis regression in patients with fibrotic liver disease[330]. There is also strong interest in understanding the role of risk stratification, based on parameters related to liver stiffness or genetic markers, in the design of clinical trials[330]. For example, some data suggest that milder fibrosis (F1–F3) will regress naturally, whereas more advanced fibrosis or cirrhosis will require antifibrotic drug interventions.

More than 100 drugs are being tested in liver fibrosis, with approximately ten in phase II/III clinical trials[331] (Table 3). Most therapeutics in clinical trials aim to reduce the metabolic and/or inflammatory impact of liver disease, instead of acting directly as antifibrotic agents[324]. Agents that target fibrotic mechanisms include those that target HSC activation and proliferation, ECM degradation and/or fibrogenic signals[79,324]. Because of the rising incidence of MASLD/MASH, many candidate drugs, including PPAR agonists, FGF15/FGF19 and farnesoid X receptor (FXR) agonists, are aimed at prevention or reversal of fat accumulation in hepatocytes and normalization of insulin signalling and glucose homeostasis[331,332].

Recently, resmetirom (in conjunction with diet and exercise) became the first drug to be approved by the FDA for the treatment of MASH. Resmetirom is a thyroid hormone receptor-β agonist that promotes fatty acid oxidation, acting indirectly as an antifibrotic in patients with F1–F3 MASH[333]. The efficacy of resmetirom for the treatment of fibrosis in MASH, targeting metabolic perturbations in MASH rather than fibrotic lesions per se, holds promise for the additional candidate drugs for MASH that target metabolic or inflammatory perturbations.

Weight reduction is also an important potential therapeutic target for MASLD/MASH, including traditional diet and exercise approaches or pharmacological treatments such as glucagon-like peptide (GLP1) receptor agonists[331,332]. Of note, although many of the therapeutics being developed for hepatic fibrosis — for example, resmetirom — target lipid homeostasis, it is unlikely that these drugs will be effective in fibrosis in other organs that do not involve the injurious effects of steatosis.

Conclusions and perspectives

Chronic disease driving tissue injury and ultimately fibrosis is a substantial burden for patients and health-care systems. After an initial insult probably characterized by epithelial and/or endothelial damage, signalling pathways converge to a central fibrosis response that is shared across organs. Part of this shared pathological pattern is the association between the presence of inflammation and fibrosis. Although fibrosis is a consequence of essentially all chronic immune-mediated diseases, there are distinctive features in terms of the intensity and presence of the inflammatory process across organs. Examples include the very severe, relapsing–remitting and tissue-destructive nature of inflammation in IBD and in certain glomerulonephritides, which contrasts with the less intense but constant inflammation that is characteristic of CKD, skin or lung fibrosis.

Such apparent differences in the temporal relationship between inflammation and fibrosis across different organ systems may be influenced by biased patient phenotyping or sampling at the time of disease presentation, but this remains unclear, as so far, the scientific community has not made a serious attempt to characterize these relationships. As a starting point, we have collected types of experimental fibrosis model from different organs (Supplementary Table 1). We strongly encourage deployment of advanced analytics on genomic and scRNAseq or snRNAseq information from these models or similar data generated by fibrosis researchers. Today, these data are essentially confined in isolated pockets of specialty medical information. Is it possible to create an AI model that would point towards conserved versus organ-specific molecular pathways? What could such an unsupervised AI model tell us about cell types in the fibrotic niche across

organs? What if we impose constraints such as conserved interactions between epithelial cells and fibroblasts or between macrophages and fibroblasts? What can we learn if a time course element is included? Can we identify gene 'signposts' that would allow lessons learned from these preclinical models to be applied to humans? Although this may appear to be a purely academic effort, we believe that this could have direct implications for the discovery of next-generation therapeutics and their evaluation in clinical trials.

Antifibrotics in certain diseases, such as IBD, may need to be combined with anti-inflammatory medication to effectively prevent progression and/or elicit regression of fibrosis. It remains to be determined whether such an approach is applicable to other organs for which this concept does not currently have acceptance, such as the kidney. Furthermore, it needs to be explored whether there is a cross-organ immune signature associated with fibrosis, and whether this signature could be exploited to reverse fibrosis. Conversely, the presence of inflammation may be necessary for effective reversal of fibrosis. This is exemplified by myeloid cell subtypes that drive fibrosis early in disease but retain the ability to degrade deposited ECM and hence could potentially be required for fibrosis resolution later on. The community should explore whether such a protective immune phenotype can be detected across different organs.

Although many candidate drugs intended to directly target mechanisms of fibrosis have shown efficacy in preclinical models, far fewer have progressed to clinical testing, and only pirfenidone[334] and nintedanib have received FDA approval for the treatment of IPF, and nintedanib has also been approved for SSc-ILD and progressive fibrosing ILDs[305,335]. Pirfenidone has been investigated also in renal fibrosis. The drug was shown to slow renal function decline in focal segmental glomerulosclerosis[280] and diabetic kidney disease[281], but further clinical development has been hampered by its safety profile in such a chronic therapy setting.

These human clinical observations were supported by both pirfenidone[222] and nintedanib[223] functionally inhibiting an activated mesenchymal phenotype, a common mechanism of fibrosis across organs (Box 2). Activated mesenchymal cells are characterized and defined by their ability to produce ECM. The field is just beginning to understand mesenchymal cell heterogeneity and plasticity to further define the activated mesenchymal cell phenotype, aided by insights from single-cell technologies (Box 1). We believe that the discovery of approaches to selectively target these cells in the context of a profibrotic ECM environment should be a research priority.

Another untapped opportunity is to address early mechanisms of fibrosis shared across organs, such as the spatiotemporal relationship of epithelial–mesenchymal interactions, which is relevant in all organs, including the skin[336] (although not in SSc). Despite its promise, no candidates specifically addressing the biology of the epithelial–mesenchymal interface in fibrosis have yet progressed into

Box 2 | Common and organ-specific features or pathological mechanisms of fibrosis

Common features and mechanisms

- Transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), developmental pathways such as Hedgehog, WNT, Notch, matrix metalloproteinases and tissue inhibitors of metalloproteinase as central mediators of the fibrotic process
- Epithelial–mesenchymal transition (EMT) or endothelial–mesenchymal transition (EndMT) as a potential mechanism for modulation of epithelial cells to activated fibrotic cells (controversial)
- Myofibroblasts defined by acquisition of positivity for α-smooth muscle actin, although it has become clear that these α-smooth muscle actin-expressing fibroblasts are often not the cells that produce the most extracellular matrix (ECM) and thus various other fibroblast populations are involved in fibrosis progression
- Interactions between macrophages and fibroblasts
- Spatiotemporal relationship of epithelial–mesenchymal interaction
- Key cytokine mediators such as IL-6, IL-13 and IL-10
- Vascular injury and dysfunction is a key component of fibrosis and organ functional decline

Organ-specific features and mechanisms

Lung

- Loss of terminal small airway epithelial cell subpopulations with replacement by highly secretory hyperplastic epithelial cells
- Development of fibroblastic foci, representing buds of activated fibroblasts and myofibroblasts
- Distinct pathogenic roles for resident macrophages and recruited circulating monocytes

Intestine

- Creeping fat postulated as potential modulator of gut strictures

Kidney

- Proteinuria with subsequent tubule epithelial injury is a key driver of inflammation and fibrosis
- Perivascular fibroblasts and pericytes contribute to the vast majority of ECM-producing cells and their detachment from capillaries triggers capillary loss and subsequent kidney functional decline

Skin (systemic sclerosis)

- Fibrotic niche with inflammation and autoimmune components (B cells, T cells, dendritic cells)
- Specific injured endothelial cell phenotype
- EndMT rather than EMT might contribute to some extent to the fibrotic process

Liver

- Apoptotic epithelial cells as fibrotic stimulus
- Origin of activated myofibroblasts as hepatic stellar cells
- Retinyl ester storage
- Steatosis, abnormal lipid metabolism, adipose–liver interactions
- Perturbations in portal, central and sinusoidal endothelial cells contribute to hepatic fibrosis

clinical development. Epithelial cell phenotypes have been successfully targeted in oncology with drugs that focus on functionally impairing or depleting them, such as EGFR inhibitors (for example, afatinib, dacomitinib, erlotinib and gefitinib). However, the intervention required in fibrosis may be the opposite: an approach that protects epithelial cells from the diverse insults that they are exposed to, such as proteinuria, pathogens or cigarette smoke. Two main factors impede the progress of novel drugs in this space: the potential risk of initiating cancer when inducing epithelial cell activation or proliferation, and the likely lengthy clinical trials requested to test this hypothesis. Potential insights to address this challenge could be gained by collaborating with researchers in oncology, where failure to exploit comparable pathobiological mechanisms has hampered success in stromal cancers.

In addition to common fibrotic mechanisms, there are organ-specific processes and mechanisms (Box 2) that may be exploited as a starting point for the development of drugs that could at least partially attenuate the fibrotic process. Prototypical examples of this approach include ACE inhibitors and/or angiotensin II receptor blockers[337] and SGLT inhibitors[338] or GLP1 receptor agonists[339], reducing proteinuria, a hallmark of renal tubule epithelial injury, which results in a reduction in inflammation and fibrosis within the renal parenchyma that translates into preserved renal function.

Overall, a disconnect remains between our understanding of fibrotic mechanisms in human disease and our ability to translate these insights into effective drugs. Addressing this disconnect will require refinement of preclinical target evaluation, including prioritization of animal models that have a higher likelihood to predict response in clinical trials, or at least have a much more granular characterization of which disease pathways are active in them so as to be able to pair an intervention with a particular mode of action to the appropriate mechanistic model. Complex ex vivo systems such as organ-on-a-chip or mini-organs that harness primary human cells in an ECM environment from healthy and diseased tissues might be a complementary tool for evaluation of early-stage compounds.

Efficiency in clinical trials needs to be increased by identifying precise and validated predictors of fibrotic disease progression to enrich for patients with high-risk phenotypes[340]. Moreover, antifibrotic drug development currently requires lengthy clinical trials, which are often prohibitively costly. Biomarkers for early response to therapy, derived from cross-sectional imaging or tissue samples, need to be developed as surrogate end points. However, for most organs, the absence of a treatment of known efficacy currently makes complete biomarker validation challenging.

Furthermore, the fibrosis field lacks a marker of the current status of profibrotic activation, regardless of the affected organ. All available markers of fibrosis, such as the degree of deposition of collagen or the number of myofibroblasts labelled by αSMA, combine the cumulative chronic damage with the current activity of the fibrotic process, without being able to separate them. We urge the community to develop tissue-based approaches to untangle these two critical elements of the disease, as done previously for instance for SLE-associated nephritis[341]. The availability of such a biomarker would potentially enable 'bucket' trials, to assess the efficacy of a single drug in patients with multiple fibrotic diseases of different organs under a single protocol.

This framework becomes even more complex considering that development and validation of clinical trial end points based on accepted methodological standards require the existence of a treatment of known efficacy. However, for most fibrotic diseases, treatments of known efficacy are lacking. To resolve this vicious cycle of no antifibrotic therapies and no validated end points, the field of fibrosis needs to rethink and coalesce around a global approach involving scientists, industry leaders, patients and regulatory partners. After systematically reviewing existing studies, a consensus (for example, using RAND methodology) could provide the basis for the items for index development. Contemporary methodological standards need to be applied for assessment of validity, reliability and responsiveness. Such an attempt has proved effective in liver and intestinal fibrosis[255,342].

None of the above challenges is novel, and comparable obstacles have been observed and overcome for other diseases such as viral hepatitis, cancer or HIV, which are also all examples in which combination therapy proved to be part of the solution. Global translational consortia are forming to support addressing these challenges (Supplementary Table 2). Fibrogenesis is complex, dynamic and multifactorial, and the expectation that targeting one single mechanism could fully prevent or treat this disease manifestation is likely to be naive. Future antifibrotic approaches will probably address multiple parallel mechanisms simultaneously and require adaptation throughout the course of therapy based on biomarker profiles.

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Author contributions

All authors contributed to discussion of the content and wrote sections of the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

F.R. is on the advisory board of or consultant to Adiso, Adnovate, Agomab, Allergan, AbbVie, Arena, AstraZeneca, Bausch & Lomb, Boehringer-Ingelheim, Celgene/BMS, Celltrion, CDISC, Celsius, Cowen, Eugit, Ferring, Galapagos, Galmed, Genentech, Gilead, Gossamer, Granite, Guidepoint, Helmsley, Horizon Therapeutics, Image Analysis Limited, Index Pharma, Landos, Janssen, Koutif, Mestag, Metacrine, Mirum, Mopac, Morphic, Myka Labs, Organovo, Origo, Palisade, Pfizer, Pliant, Prometheus Biosciences, Receptos, RedX, Roche, Samsung, Sanofi, Surmodics, Surrozen, Takeda, Techlab, Teva, Theravance, Thetis, Trix Bio, UCB, Ysios and 89Bio. J.H.W.D. has consultancy relationships with and/or is part of the speaker or advisory board of AbbVie, Active Biotech, Anamar, ARXX, AstraZeneca, Bayer Pharma, Boehringer-Ingelheim, Calliditas Therapeutics, Celgene, Galapagos, Genentech, GSK, Inventiva, Janssen, Novartis, Pfizer, Roche and UCB. J.H.W.D. has received research funding from Anamar, Argenx, ARXX, BMS, Bayer Pharma, Boehringer-Ingelheim, Cantargia, Celgene, CSL Behring, Galapagos, GSK, Inventiva, Kiniksa, Lassen, Sanofi-Aventis, RedX and UCB. J.H.W.D. is CEO of 4D Science and Scientific Lead of FibroCure. R.K. is founder and shareholder of Sequantrix GmbH and has grants from Travere Therapeutics, Galapagos, Chugai, AskBio and Novo Nordisk and is a consultant for Bayer, Roche, Chugai, Pfizer, Novo Nordisk, Hybridize Therapeutics and Gruenenthal. T.M.M., via his institution, has received industry-academic funding from AstraZeneca and GlaxoSmithKline R&D; and consultancy or speaker fees from AstraZeneca, Bayer, Boehringer-Ingelheim, BMS, CSL Behring, Fibrogen, Galapagos, Galecto, GlaxoSmithKline, IQVIA, Merck, Pliant, Pfizer, Qureight, Roche, Sanofi-Aventis, Structure Therapeutics, Trevi and Veracyte. M.P. is an employee of Medicxi Ventures. L.E.N. and B.H. declare no competing interests.

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